Local membrane versus systemic consequences of peritoneal dialysis treatment

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Declaration Part 1. To be bound in the thesis

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Abstract

The primary intent of this thesis is to delineate the relative roles of local membrane and systemic consequences of peritoneal dialysis therapy, with particular reference to the role of inflammation and a severe, uncommon complication, encapsulating peritoneal sclerosis (EPS). Data sources comprised observational cohort studies as well as registry data: the Stoke PD study, a single centre study with clinical data, the Global Fluid Study (GFS), a multinational study with clinical data and repeated dialysate and plasma samples, and Scottish Renal Registry (SRR) and AnzData registry data.

Through a cross sectional analysis of dialysate and plasma samples from GFS for inflammatory cytokines, we demonstrated that peritoneal and systemic inflammation are mostly separate processes although there is an association for IL-6 along with a steep concentration gradient from dialysate to plasma. Peritoneal inflammation, though IL-6, is the strongest determinant of peritoneal solute transport, and systemic inflammation, though IL-6, is a strong predictor of patient survival although peritoneal may contribute to systemic inflammation.

Through a nested case control study of GFS we showed that inflammatory cytokines are upregulated within the peritoneum prior to developing EPS. With a nested case control design from the Stoke PD study, we showed that a decrease in ultrafiltration, likely due to increased fibrosis causing a reduction in osmotic conductance to glucose, also predisposes to EPS. A competing risks analysis of SRR and AnzData showed that patients at a high risk of death, have a low risk of EPS. These findings provide supporting evidence for the theory that the risk of EPS develops through the accumulation of inflammation-driven fibrosis due to dialysate exposure over a long period of time.

Dialysate contains high concentrations of glucose and absorption of this drives impairment of systemic glucose metabolism, demonstrated through a cross sectional analysis of GFS.

Abbreviations

λ	Hazard Function
Λ	Cumulative Hazard Function
APD	Automated Peritoneal Dialysis
AR	Appearance Rate
AQP	Aquaporin
BMI	Body Mass Index
CAPD	Continuous Ambulatory Peritoneal Dialysis
CIF	Cumulative Incidence Function
CKD	Chronic Kidney Disease
Cr	Creatinine
CRF	Case Report Form
CRP	C-reactive Protein
D/D0	Drained dialysate glucose/initial dialysate glucose concentration ratio
D/P Cr	Dialysate/Plasma Creatinine Ratio
DM	Diabetes Mellitus
ECL	Electrochemiluminesence
ELISA	Enzyme-Linked Immunosorbent Assay
EPS	Encapsulating Peritoneal Sclerosis
ESRF	End Stage Renal Failure
GDP	Glucose Degradation Product
GFS	GLOBAL Fluid Study
h	Sub-distribution Hazard Function
Hb	Haemoglobin
HD	Haemodialysis
Ico	Icodextrin

IFN-γ	Interferon-gamma
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
Kt/V	Measure of urea clearance
PD	Peritoneal Dialysis
PD-CRAFT	PD-Competing Risks Analysis for Long Term Outcomes Study
PDC	Personal Dialysis Capacity
PDDB	Peritoneal Dialysis Database
PET	Peritoneal Equilibration Test
PSTR	Peritoneal Solute Transport Rate
RRF	Residual Renal Function
S	Survival Function
SPA	Standard Permeability Analysis
TNF-α	Tumour Necrosis Factor alpha
UF	Ultrafiltration
Ur	Urea
VEGF	Vascular Endothelial Growth Factor
VIF	Variance Inflation Factor

Publications Arising To Date

Original Articles

Chapter 4: Independent Effects of Systemic and Peritoneal Inflammation on Peritoneal Dialysis Survival.

Lambie M, Chess J, Donovan KL, Kim YL, Do JY, Lee HB, Noh H, Williams PF, Williams AJ, Davison S, Dorval M, Summers A, Williams JD, Bankart J, Davies SJ, Topley N; on behalf of the Global Fluid Study Investigators.

J Am Soc Nephrol. 2013 Sep 5. [Epub ahead of print]

Chapter 7: The peritoneal osmotic conductance is low well before the diagnosis of encapsulating peritoneal sclerosis is made.

Lambie ML, John B, Mushahar L, Huckvale C, Davies SJ.

Kidney Int. 2010 Sep;78(6):611-8. doi: 10.1038/ki.2010.186. Epub 2010 Jun 23

Commentary based on work in thesis

Chapter 8: Towards standardized reporting in studies of encapsulating peritoneal sclerosis.

Lambie M, Braun N, Davies SJ.

Perit Dial Int. 2013 Sep;33(5):482-6. doi: 10.3747/pdi.2013.00165

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1 Aims

Peritoneal Dialysis is a commonly used treatment for end stage renal failure, but its use is limited by the high complication rate, including issues related to peritoneal membrane damage. The aim of this thesis is to explore the relationship between local and systemic complications of peritoneal dialysis, with particular reference to inflammation and the role of this in peritoneal damage as demonstrated by changes in physiological measures. As a severe complication of PD, encapsulating peritoneal sclerosis will be investigated with particular attention, to explore the hypothesis that peritoneal inflammation and subsequent fibrosis drives the increase in risk of EPS associated with time on PD. This will be considered in several sections.

1 –The interactions between peritoneal and systemic inflammation, patient survival and peritoneal solute transport rate (Chapter 4).

2 –Changes in peritoneal solute transport rate and peritoneal and systemic inflammation over time (Chapter 5)

3 –The role of peritoneal and systemic inflammation in driving encapsulating peritoneal sclerosis (Chapter 6)

4 – Changes in the peritoneal membrane physiology preceding encapsulating peritoneal sclerosis (Chapter 7)

5 –Describing the risk factors for encapsulating peritoneal sclerosis, in the context of the high mortality rate of patients on peritoneal dialysis (Chapter 8)

6 – Investigating the effect of dialysate glucose on systemic metabolism (Chapter 9)

2 Background

2.1.1 What are the main causes of peritoneal dialysis technique failure?

Renal replacement therapy (RRT) is a very successful form of organ replacement therapy for end stage renal failure (ESRF), with a steadily increasing prevalence throughout the world. Transplantation is the form of RRT with the greatest impact on morbidity and mortality but, with a limited supply of donors, a significant transplant failure rate and with the difficulty of successfully transplanting older and more comorbid patients, there is still a need for dialytic forms of RRT. Haemodialysis is the most commonly used dialytic therapy, but there is still a significant number of patients on peritoneal dialysis (PD), and the use of this modality is increasing particularly in the developing world and in the United States due to the lower cost compared with haemodialysis. PD is where dialysate is instilled intra-peritoneally to use the peritoneal membrane as a semi-permeable membrane, allowing clearance of toxins by diffusion and clearance of water through convective flow.

Unfortunately, PD remains limited by a high technique failure rate and a high mortality rate such that, by 3 years only 30% of patients remain on PD. The causes of technique failure change with time with catheter and abdominal problems, and psychosocial problems a significant problem in the first 3 months and decreasing with time, while rates of infectious peritonitis stay relatively constant with time and the contribution of underdialysis or ultrafiltration failure increases with time.(1) Poor ultrafiltration rates are a risk for PD failure particularly in anuric patients (2) and with increasing technique survival rates, ultrafiltration failure is an increasing problem.

Encapsulating peritoneal sclerosis (EPS) is an unusual complication of PD, initially described in Japan, but now recognised as a problem within the UK and throughout Europe. It is characterized by a cocoon of fibrotic material in the peritoneal membrane which constricts the bowel, leading to obstruction and a high mortality. The main risk factor appears to be time on PD, although peritonitis has also been reported as a risk factor.

2.1.2 What are the main causes of death on peritoneal dialysis?

Both forms of dialysis have a very high mortality rate, with a massively elevated rate when compared with age, gender and ethnicity matched populations, (3) and a survival rate lower than that of many forms of cancer in the older population. Cardiovascular disease (CVD) is responsible for about 45% of the deaths in dialysis patients, although this risk increases with falling estimated glomerular filtration rate (eGFR) and is not specific to dialysis. (4) There is some evidence that PD is associated with an increased hazard of cardiovascular death compared with haemodialysis. (5) Infection is responsible for about 20% of deaths, withdrawal of dialysis 15% and malignancy 7%, although a significant number of patients have an unclear cause of death.

Although Framingham risk factors are more prevalent in dialysis patients, including age, hypertension, diabetes, dyslipidaemia and physical inactivity, these do not account for all of the increased risk. Other factors identified as risk markers for CVD-related death include anaemia, hyperparathyroidism, hyperphosphataemia and vascular calcification, oxidative stress, inflammation and malnutrition, elevated asymmetric dimethylarginine, hyperhomocysteinaemia, endothelial dysfunction and accumulation of advanced glycation end products.

2.2 What is our current understanding of peritoneal membrane function?

2.2.1 What is the structure of the peritoneal membrane?

The peritoneum is a serous membrane that lines the abdominal cavity. The outer layer is known as the parietal peritoneum and the inner layer that wraps around the abdominal organs is the visceral peritoneum. Around 80% of the peritoneum is visceral and the blood supply is derived from the mesenteric arteries and drains into the portal veins, the other 20% is parietal which gets its vascular supply from the abdominal wall. Despite the visceral peritoneum having the larger proportion of the total surface area, rat models have suggested that the parietal peritoneum has more contact with dialysate, suggesting that it is the parietal peritoneum that is most important to peritoneal transport, (6) supported by studies of 15 patients post-omentectomy (7) and of dogs post-evisceration, omentectomy or mesenterectomy (8) where no difference was found in solute transport**.**

Table 2-1: Layers of Parietal Peritoneum from Superficial to Deep

Layer	Characteristics
Mesothelium	Flat, single cell layer around 0.5mm thick. Covered in microvilli.
Basement Membrane	Mostly type IV collagen. 25-40nm thick.
Sub-mesothelial Compact Zone	Fibrous tissue, collagen and scattered elastin fibres. Around 50mm thick in non-uraemic subjects
Loose connective tissue zone	Collagen, occasional fibroblast spindle cells, mononuclear phagocytes, mature lymphocytes and adipose tissue. Also contains blood vessels, lymphatics and nerves.

Milky spots are also found, being collections of macrophages with lymphocytes and occasional plasma cells supplied by blood and lymph vessels. They are found on other serosal surfaces, and within the peritoneal cavity particularly on omentum. Most authors agree that their function is that of a secondary lymphoid organ.

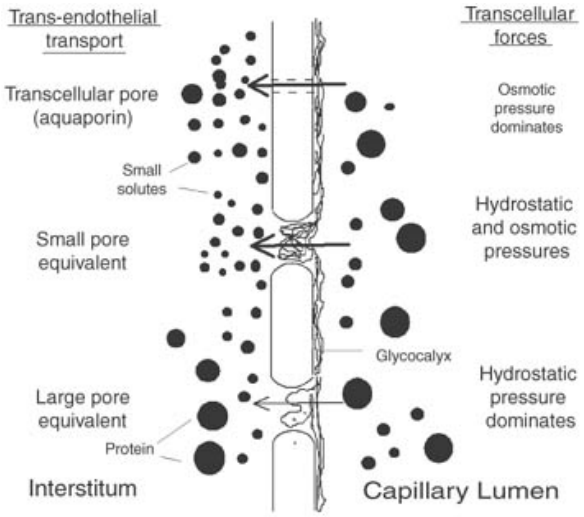
2.2.2 What theoretical models describe the solute transport and fluid transport (ultrafiltration) characteristics of the peritoneal membrane?

2.2.2.1 The three pore model

There are several possible rate-limiting steps for peritoneal diffusive transport of low molecular weight solutes: peritoneal blood flow, capillary endothelium, interstitial space or mesothelium. There is indirect evidence that the blood flow to the peritoneum is several times that of the urea clearance suggesting that this is not limiting (9) and more animal model work also found no restriction at the level of the mesothelium (10) or in a normal interstitial space. (11) This leaves the capillary endothelium as the primary site of restriction to peritoneal diffusion i.e. this constitutes the 'semi-permeable membrane'.

Evidence for diffusive solute transport across at least 2 pores came from studies of the transport of different sized molecules where the kinetics of transfer of large proteins, which are unable to pass through the small pore system responsible for the diffusion of low molecular weight solutes, indicated the presence of a smaller number of large pores. (12) This has then been adapted into a 3 pore model (figure 1) to explain the phenomenon of sodium sieving, by including an ultrasmall, water selective, pore**.** (13) Supportive evidence for this theory came with the recognition of aquaporins (14) and mouse knock outs of AQP1 confirmed the role of AQP's by preventing sodium sieving.(15) The 3 pore model has also been used to successfully predict the success of the colloid osmotic agent Icodextrin separately or in combination with glucose**.** (16) One of the limitations to this theory is the uncertainty over what exactly the theoretical small pores represent anatomically.

Figure 2-1: The 3 Pore Model of Transport Across the Peritoneal Capillary



To explain fluid transport (ultrafiltration) across the peritoneum the equation

Equation 2-1: Ultrafiltration Across the Peritoneum

$$J_v = K_f([P_c - P_i] - \sigma[\pi_c - \pi_i])$$

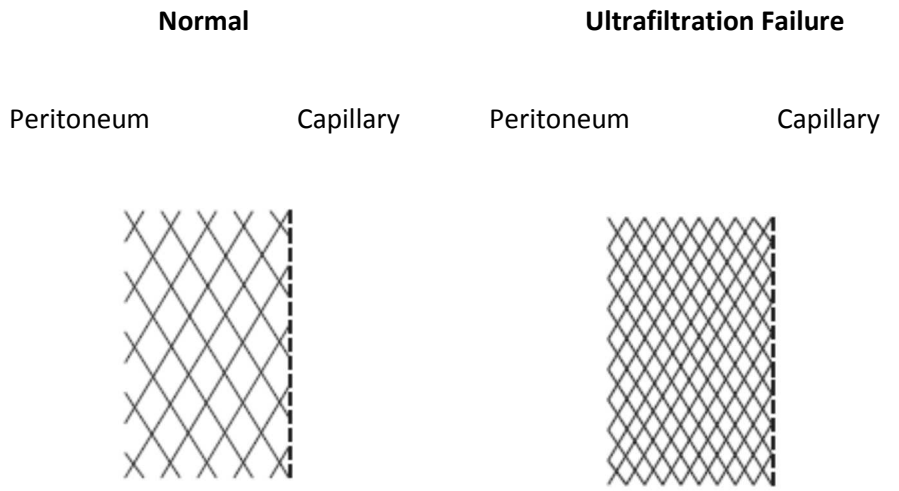
is used, where J_v is the net fluid flux across the capillary, K_f is a proportionality constant (a function of the liquid permeability of the membrane and its area), P is the hydrostatic pressure and π is the capillary and colloid osmotic pressure in capillary or interstitium, and σ is the reflection coefficient, This governs convection across capillaries and was devised by Ernest Starling in 1896.

As osmotic pressure is one of the principal determinants of water flux across the peritoneal membrane, this is utilised in PD by varying the osmotic pressure of the dialysate (usually by adjusting the glucose concentration) to increase or decrease the water flux into the dialysate. Through this technique, the fluid balance of the patients can be controlled. However, according to the 3 pore theory, glucose should diffuse across the small pores down its concentration gradient so any increase in capillary endothelial area, and thereby an increase in the small pore area, will accelerate glucose absorption, diminishing the osmotic gradient, and therefore will limit the degree of ultrafiltration that can be achieved.

2.2.2.2 The fiber matrix/3 pore model

A further development of the 3 pore model became necessary with the recognition that solute transport could become 'uncoupled' from ultrafiltration (17) i.e. that something other than solute transport affecting the osmotic gradient could adversely affect ultrafiltration in long term patients. Biopsies of peritoneal membrane from patients on PD demonstrated a substantial increase in the sub-mesothelial compact zone with time on PD**.** (18) This led to the development of a modification of the 3 pore model to include a fibrous matrix as another serial resistance, as shown in figure 2. By simulating an increased effective peritoneal surface area (i.e. perfused capillaries in contact with dialysate) in conjunction with an increased density of matrix and an increase in fiber size as would occur with the addition of collagen to proteoglycan, the uncoupling of solute transport, measured as the Mass Transfer Area Coefficient (MTAC) or the synonym Permeability-Surface Area product (PS), from hydraulic permeability (LpS) was reproduced. (19) This model has not yet been extended to predictions surrounding macromolecular transport due to the complexity of modeling this with serial resistances. By decreasing the LpS, the osmotic conductance to glucose (LpSσg) should also be adversely affected, as has been demonstrated in patients with UF failure on long term PD. (20) One study has demonstrated an association between a loosely defined group of impaired UF capacity patients on long term PD and an increased peritoneal thickness. (21)

Figure 2-2: Diagrammatic Representation of the Fiber Matrix/3 Pore Model

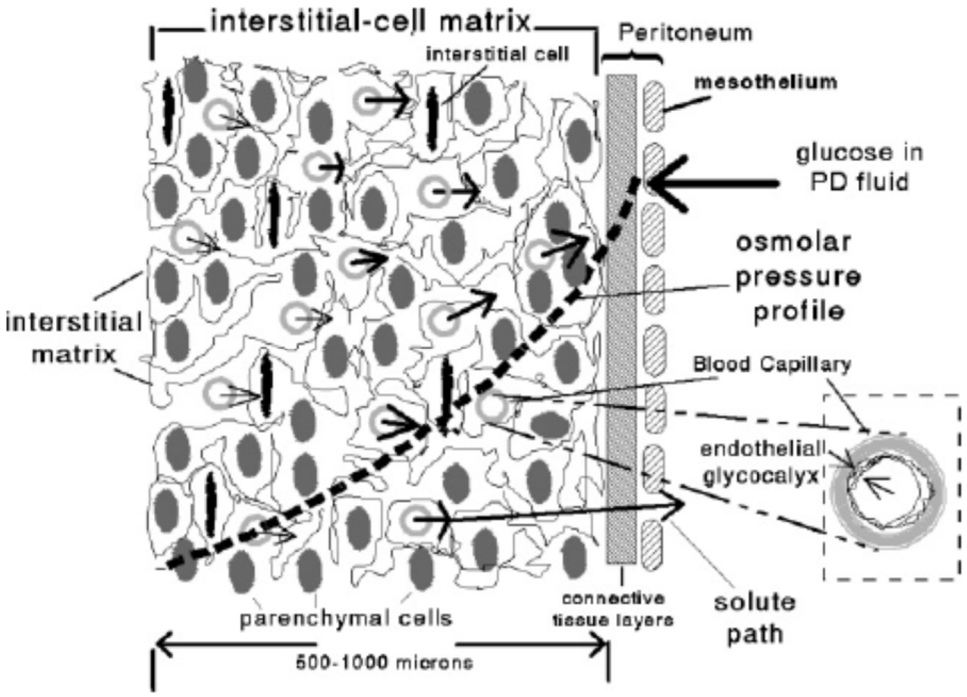


The image on the left represents the peritoneal membrane at the start of PD with a loose matrix, having little impact upon the convective flow of water on the left. On the right the image demonstrates a dense matrix representing the increase in membrane fibrosis with time on PD, causing a resistance to the free flow of water, and subsequent UF failure. The dotted line in both images represents the capillary endothelium.

2.2.2.3 The distributed model

Another model has been developed to take account of the variable depth within the membrane of the peritoneal capillaries, with an osmotic gradient developing from the maximum at the mesothelium down to the lowest levels at the greatest depth within the membrane (figure 3). This is mathematically more complex, with the solution requiring partial differential equations with several variable parameters, (22) and because of this added complexity with no major clinical benefit, it has not been widely adopted but remains a research tool.

Figure 2-3: Diagrammatic Representation of the Distributed Model With a Variable Capillary Depth and Exponentially Decreasing Osmotic Pressure



2.2.2.4 How do we measure peritoneal function?

As PD is primarily trying to achieve both solute removal through diffusion into dialysate, and water removal through manipulation of the osmotic pressures, it is useful to measure the capacity of the membrane to deliver these. Several tests have been devised do this in a clinical setting, including the personal dialysis capacity (PDC) test, and the standard peritoneal permeability analysis (SPA), but the simplest and most widely used is the peritoneal equilibration test (PET). (23)

The simplified PET consists of administration of a standard glucose based dialysate (either 2.27% or 3.86%) and leaving in situ for 4 hours. The ratio of the dialysate to plasma creatinine concentrations (D/P Cr) at 4 hours is primarily a measure of how much diffusion has occurred. As diffusion is an inactive process, the ratio cannot rise above 1. Diffusion of solutes is mostly across the small pores, so a change in D/P Cr represents a change in small pore area, and thereby it is a measure of effective

peritoneal membrane area. D/P Cr is not a pure measure of diffusion, as solute drag will occur with convection, but the proportion is relatively small and the methods to measure diffusion only (Mass Transfer Area Coefficient - MTAC) are more complicated so D/P Cr is used as a close approximation. The original paper describing the PET divided the patients into groups (High, High-Average, Low Average and Low) based upon the D/P Cr, but this does change a continuous variable into a categorical variable.

To measure the amount of ultrafiltration achieved, the volume of dialysate drained is measured, and the initial volume subtracted (with adjustments for bag overfill as necessary). This is called the ultrafiltration (UF) capacity, and it represents a composite measure, reflecting both the liquid permeability (Lp) and the surface area (S) of the membrane, the effective lymphatic reabsorption, and each of the 2 pressures determining the hydrostatic pressure gradient, the colloid osmotic pressure gradient and the crystalloid osmotic pressure gradient, itself partly determined by the reflection coefficient of glucose (σ_g).

As UF capacity is a lumped parameter, newer tests, such as the double mini PET (24) or the sequential PET, (25) have been devised which allow calculation of the more specific osmotic conductance to glucose ($LpS\sigma_g$). The advantage of this calculation is that, as σ seems unlikely to change with time on PD, $LpS\sigma_g$ should better reflect any changes that occur in the underlying liquid permeability to water (LpS) of the peritoneal membrane but as the protocol for this is more complicated that the simplified PET it has not yet been widely adopted.

2.2.3 What do we know of the predictors of solute transport and ultrafiltration?

2.2.3.1 Solute transport

2.2.3.1.1 Early changes

In the largest study of solute transport in incident (<6 months) patients, using 3,188 registry subjects from Australia and New Zealand, the independent predictors of faster solute transport were

diabetes, lower BMI, male gender, having received APD and ethnicity. (26) This study was confounded by incomplete reporting which was unlikely to be random as the groups with and without data were significantly different. As body surface area, and therefore presumably peritoneal surface area, is proportional to height (in different formulae, to different powers between 0 and 1), while BMI is inversely proportional to height squared, the association in this study seems likely to be due to a mathematical coupling with body surface area.

Another study of the predictors of incident peritoneal transport was performed on data from the CANUSA study, where 14 centres providing 680 patients had 606 with details of the incident PET test. (27) Multivariable analysis was not used, but age, gender, diabetic status and serum albumin were significantly different amongst different categories of peritoneal transporter status. Neither of these two multicentre studies were analysed with techniques taking possible centre effects into account.

The third large study of peritoneal transport at the start of dialysis was a single centre study with 574 patients (17) where multivariable analysis established male gender, lower plasma albumin and a larger urine volume as predictors of faster transport, although with an R^2 value of only 0.215. Comorbidity, according to the Stoke comorbidity index, and age were not significantly associated. Hypoalbuminaemia is predictably associated with solute transport as albumin will cross small pores as well as through large pores so a larger effective membrane area will increase dialysate albumin losses. A Danish study (28) confirmed the association between large pore flux (Jv_L from the Personal Dialysis Capacity test) and subsequent hypoalbuminaemia, but a study in Canada (29) found an association between the pre-dialysis albumin and the subsequent transport status (D/P Cr), suggesting there might be more than one cause for this association e.g. systemic inflammation increasing solute transport, and decreasing albumin levels through its role as a negative acute phase protein.

There is significant variability in incident solute transport only partially explained by the factors above, and these factors vary between studies. One explanation may lie in the timing of the PET test in the different studies. Johnson et al found a significant increase in solute transport occurred between 1 and 4 weeks, but reasonable correlation between measurements at 4 weeks and 1 year.(30) In this study some informative censoring did occur between 4 weeks and 1 year with those patients not surviving on PD to 1 year having a greater increase in solute transport within the first month. An apparently contradictory result was found by Struijk et al (31) where there was a decrease in solute transport from 1 month to 5 months. This could potentially be explained by an initial reaction to glucose exposure inducing vasodilation with an increase in perfused capillaries, followed by an adaptation to chronic glucose exposure although some longitudinal studies found either stability in solute transport over this time period, or a fall in solute transport occurring later. (32) These results are all prone to informative censoring as a fast solute transport predicted both technique and patient survival during the period when the majority of these studies were performed.

The significance of these early alterations in solute transport is unclear as Davies found a lack of alteration in UF capacity. The study by Davies also examined the early changes, finding that a change in D/P Cr of <0.1 in the first 6 months was within normal test variation and not predictive of subsequent increases in solute transport, but a change of >0.1 was. This is potentially explained by the decrease in UF caused by an increase in absorption of dialysate glucose being matched by an increase in the osmotic conductance to glucose through an increase in surface area.

Other factors may contribute to the variability in solute transport. La Milia et al conducted a non-randomised study comparing pre-dialysis PET tests with those at 4 and 16 months, and they demonstrated an increase in D/P Cr in CAPD patients, not found in APD patients suggesting an effect of modality early on. (33) Furthermore, the definition of the commencement date of PD is not clear

in most studies, possibly interpreted as the start of training or at the end of a variable length of training with varying amounts of dialysate exposure.

Another reason for the variability in predictors of solute transport between studies was suggested in an editorial by Davies. (34) Solute transport predictors can interact with each other e.g. BMI, age and diabetes, and with other factors such as ethnicity, such that the case mix for each study, in combination with different statistical methods, may well contribute to the different findings.

Several small studies have also found genetic associations with incident solute transport including polymorphisms in endothelial nitric oxide synthase (eNOS), (35) in RAGE but not eNOS or VEGF, (36) in IL-6, but not VEGF or eNOS, in a European population (37) and in IL-6 in a Korean (38) population. As a genetic predisposition to IL-6 production is consistently associated with faster solute transport, this strongly suggests that inflammation partially determines solute transport. Interestingly, the level of change in PSTR at 12 months was found to be associated with VEGF (39) and IL-10 (40) polymorphisms, when these did not affect baseline values, adding weight to the theory that the drivers of PSTR change over time.

2.2.3.1.2 Late changes

The investigation of membrane function in prevalent patients is essentially the study of longitudinal changes with duration of PD and there are several potential drivers of change with time – peritonitis episodes, the presence of a catheter, bio-incompatible dialysate, and uraemia. Most studies have found a consistent increase in solute transport with time on PD, (31,41,42) although there were differences in when this rise commenced which could be linked with the differences between centres in early changes discussed above. The rise in solute transport indicates an increase in perfused capillaries. There does appear to be an increase in capillary area with solute transport [40]but there have been no longitudinal studies showing a clear relationship between capillary density and time. The largest biopsy study did find greater vessel density in patients with histological

fibrosis or 'membrane failure', (18) suggesting that increases in solute transport may reflect variable levels of vasodilatation and angiogenesis.

2.2.3.1.3 Drivers of change

Peritonitis does have an effect upon solute transport linked to the number of episodes, the organism involved, and the severity of the inflammation**.** (41) This effect becomes less significant with time as the patient group without peritonitis also has a long term increase in solute transport which 'catches up' with the peritonitis group. This finding is consistent with a number of other studies, (43–45) although these studies are smaller and/or shorter.

High osmolarity, high glucose concentrations, buffer composition, pH and glucose degradation products have all been identified as components in the well recognised bio-incompatibility of conventional dialysis fluids driving impaired immune function, angiogenesis and fibrosis in animal models and in *in vitro* work. (46,47) Newer solutions designed with a more optimal bicarbonate/lactate buffer, higher pH and lower GDP content have been shown to be beneficial in laboratory studies (48) although, until recently, no benefit had been consistently identified in human randomized controlled clinical trials**.** The BALANZ trial was a study of 185 incident patients, randomized to either standard or biocompatible dialysate fluid (both from Fresenius Medical Care). The primary outcome, the rate of decline in residual renal function, just failed to meet pre-specified statistical significance, (49) but numerous secondary outcomes were statistically significant, some highly. This included the D/P Cr which was significantly faster in the biocompatible group at 1 month into the trial but this measurement remained stable. This contrasted with the standard dialysate group where the D/P Cr increased significantly over the 24 months of the trial to end up significantly faster than the biocompatible group. (50)

The effects of other dialysis solutions have not been so well studied. Icodextrin usage, which may be associated with greater intra-peritoneal inflammatory cytokine and fibrin production, (51–53) has been associated with less functional change in peritoneal membranes, albeit in an observational

study. (54) Unfortunately, none of the randomized controlled trials of Icodextrin alone have examined the effects on PSTR. One randomized controlled trial where patients were allocated to a regime of either Icodextrin for the overnight dwell with a biocompatible glucose solution and an amino acid solution (intervention group) during the day or a regime of standard glucose solutions for all dwells (control group) examined dialysate markers and PSTR changes. (55) Dialysate from the long dwell showed that the Icodextrin dwell was associated with higher IL-6 levels as well as CA125 and adiponectin, whilst serum IL-6 and CRP were no different. The PSTR in this study was significantly faster in the intervention group by the end of the 12 month follow up (0.78 ± 0.13 vs 0.68 ± 0.12, p=0.001) despite a much larger glucose load in the control group (33.7 grammes/day ±9.9 vs 130.3 ± 34.7, p<0.001).

The level of membrane exposure to glucose has also been linked with changes in the PSTR, (56) in an observational study where higher glucose exposure predated increased PSTR, even when RRF is not relevant as a confounder.(54) Whether higher glucose exposure still predicts increases in PSTR in biocompatible fluids is not known, but a study of this would help to identify the relative roles of osmolality and glucose versus GDPs, buffer composition and pH.

There are several studies in rat models suggesting an inflammatory foreign body reaction to the implantation of a catheter with effects upon angiogenesis, fibrosis and solute transport, although the applicability to humans is unclear with a different ratio of catheter to peritoneum size, and therefore a different level of peritoneal 'catheter exposure'. (57) Evidence supporting the concept of catheters causing chronic inflammation comes from observational data in haemodialysis patients showing that intravascular catheters and arteriovenous grafts are, even in fully adjusted models, associated with increased systemic IL-6 and CRP compared to arteriovenous fistulae, and that transitions from fistulae to catheters are associated with an increase, as well as vice versa. (58)

Uraemia also must have an effect upon membrane structure as evidenced by the peritoneal changes documented at the start of PD, (18) although the ongoing effect of this once PD has commenced is not known.

2.2.3.2 Ultrafiltration

2.2.3.2.1 Predictors of ultrafiltration capacity

Assessment of the predictors of UF capacity is significantly more difficult as it is a lumped parameter, reflecting the effects of intraperitoneal pressure, sump volume, effective lymphatic absorption rate, hydraulic permeability, and glucose reflection coefficients, and the measurement itself has a significant coefficient of variability of 20 to 25%**.** (34) This complexity is demonstrated by studies where the initial rate of UF was shown to be independent of solute transport, while the 4 hour volume was decreased by a fast solute transport (59) and where UF through the ultrasmall pores was found to be dependent solely on osmotic gradient, whilst small pore UF had significantly more variation. (60)

Consequently, most studies have focused on studying the effect of these experimentally derived factors and there have been few studies of sufficient size to identify clinical predictors of initial UF capacity. One exception to this was a smaller study of 367 incident patients where age was identified as predictive of UF capacity, independently of glucose concentration and solute transport (61) although this was not found in a larger study**.** (42) There have been no studies investigating the correlation between age and the size of the sub-mesothelial compact zone.

Intraperitoneal pressure is simply measured by examining the height of the column of dialysate within the dialysis catheter from the mid-axillary line, and an increase of 1cm has been shown to correlate with decrease in UF at 2 hours of 70mls**.** (62) A potentially important consequence of this association is that UF could be lower when upright compared with recumbency, but this effect seems to be small**.** (63) This effect on UF may be mediated by an increase in effective lymphatic

absorption, according to a study by Imholz et al (64) although theoretically it should have a direct effect opposing UF for a given osmotic gradient**.**

There is considerable debate within the literature regarding the true significance of the effect of lymphatic absorption, (65,66) with results from the measurement of Dextran absorption suggesting an important effect, (20,60,67) but when radiolabelled albumin was used the effective lymphatic absorption rate appeared significantly less. (68) There is some evidence that a higher effective lymphatic absorption rate may be important in UF failure occurring early in the course of PD (20) and there are experimental reasons to suspect that abnormalities may exist (vide infra).

One of the predictions of the 3 pore model was of the efficacy of colloids when trying to achieve sustained UF, borne out with studies of Icodextrin. (69) Colloids are only be absorbed through lymphatic channels thereby preserving the osmotic gradient for longer dwells, and their high reflection coefficient across small pores ensures efficient ultrafiltration for a relatively low concentration gradient. This prediction was extended to predictions for dialysates containing both Icodextrin and glucose**,** once again borne out with clinical studies, (16,70) where the effects of colloid, by counterbalancing the intracapillary oncotic pressure driving fluid reabsorption, combined with the crystalloid to produce a significant UF.

2.2.3.2.2 Longitudinal changes in UF with time

That UF capacity falls with time is consistently documented, and some of this fall could be explained by the increase in solute transport (and therefore glucose reabsorption) that also occurs with time but a report from 2004 (17) demonstrated that after 5 years of PD, there was a disproportionate fall in UF capacity linked to poorer residual renal function and greater glucose exposure. That an explanation other than solute transport is necessary to explain changes in UF capacity is also suggested by the absence of changes in UF capacity in conjunction with early changes in solute transport. Subsequent studies have suggested that some of the long term decline in UF capacity is

due to a fall in osmotic conductance of the peritoneal membrane (20) thereby causing ultrafiltration failure.

2.2.3.3 Effect upon mortality of solute transport and ultrafiltration

There is a well-established association between solute transport and mortality, with a meta-analysis by Brimble et al combining 19 prospective and retrospective studies with over 6,500 incident and prevalent patients demonstrating a relative mortality risk of 1.15 for 0.1 increase in D/P Cr. (71) Whilst a faster solute transport for a given volume of ultrafiltration should create a beneficially greater small solute clearance, it will also be associated with a greater protein loss and thereby hypoalbuminaemia, (28) a known risk factor for mortality, as well as creating faster glucose reabsorption with subsequent lower UF and greater glucose exposure. This increase in mortality with fast solute transport is consistent with several trials which have shown no benefit in greater peritoneal solute clearance**,** (72–74) but an increase in mortality with worse UF**.** (73,75,76)

Studies that included patients on APD, a strategy which should preserve UF in fast transporters, showed a smaller increase in mortality with solute transport**,** (77) Furthermore, the studies included in the Brimble meta-analysis were mostly before the introduction of Icodextrin, a dialysate solution utilizing a colloid osmotic gradient, thereby avoiding the problem of reabsorption across small pores and preserving UF. A recent analysis of the ANZDATA registry actually demonstrated a mortality hazard ratio of 0.56 for patients with a fast transport status on APD, possibly due to the superior control of fluid balance in this group, whilst there was actually an increased mortality for APD use in patients with a slow transport status. (78)

2.3 What is the role of inflammation and fibrosis?

2.3.1.1 How does the peritoneal membrane morphology change with time?

As mentioned above, one of the predominant features of the peritoneal membrane change with time is the increase in the submesothelial compact zone. This change occurs before the start of PD, with uraemia responsible for an initial increase in fibrosis, but this fibrosis increases with time on PD such that the median thickness of the compact zone is 50µm, 140µm and 700µm in normal subjects, uraemic subjects and patients on PD for over 8 years respectively. This increase in fibrosis is predominantly in the parietal peritoneum with a median thickness of 505µm (normal subjects 50µm) compared with only 20µm in the visceral peritoneum in PD patients. (79)

Figure 2-4: Sub-mesothelial compact zone thickness with time on PD

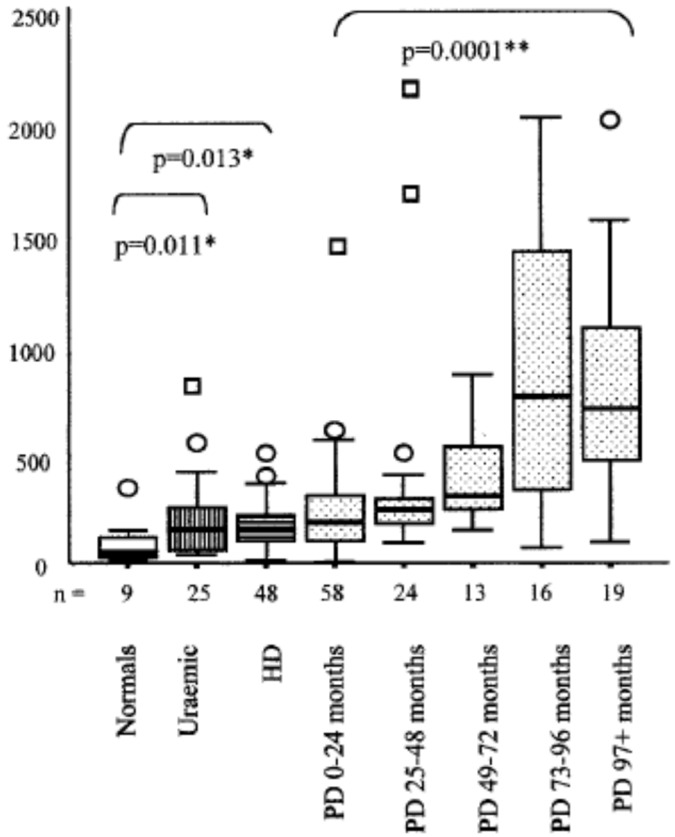


Figure from Williams et al, JASN 2001

In PD patients, the main other change described in the peritoneal membrane is a hyalinising vasculopathy of venules, small veins and sometimes arterioles, with subendothelial accumulation that may progress to narrow or obliterate the vessel lumen. This vasculopathy increased with time on PD. There is a similarity between diabetic vasculopathy and that described in PD patients, but there was not an obvious interaction between PD and diabetes affecting the vasculopathy. (18) The vasculopathy was also found in 28% of uraemic, non-PD patients, although another study of post mortem samples from PD patients with severe hyalinising vasculopathy found no evidence of vasculopathy elsewhere. (80) Again, differences are found between peritoneal surfaces with the greatest vascular changes in the parietal peritoneum. (79)

The vessel density did not increase with time on PD but it was greater in those with fibrosis (compact zone >150μm) and in samples from patients with membrane failure, although the definition of membrane failure was not clear in this study (18)

The mesothelium undergoes epithelial-mesenchymal transition (EMT) (81) where it loses epithelial morphology, and cytokeratin and E-cadherin expression in conjunction with induction of the transcriptional repressor *Snail*. The mesenchymal phenotype was marked by α_2-integrin expression and acquisition of a migratory phenotype and all of these EMT changes were induced by transforming growth factor β1 (TGF-β), potentiated by IL-1β. EMT is also linked with angiogenic stimuli (82) and solute transport**.** (83)

2.3.1.2 Does uraemia affect other serosal surfaces?

Fibrinous pleuritis has been described in a post mortem study of uraemic patients**,** (84) there have been a few reports of a fibrosing uraemic pleuritis requiring decortication for which the pathophysiology remains unclear, (85) and a retrospective review of haemodialysis patients in 2007 found that the commonest cause of exudative pleural effusions was uraemic pleuritis. (86) Acute pericarditis is a well recognised complication of uraemia, but chronic constrictive pericarditis seems to be far less common**,** (87) with only 4.8% (2 of 42) of patients requiring surgery for constrictive

pericarditis having uraemia as a cause. (88) Uraemia does occasionally appear to cause significant pathology of serosal surfaces, whether inflammatory or fibrotic, although the exact prevalence of these changes has not been fully elucidated with histological studies.

2.3.2 Is there local inflammation?

2.3.2.1 Histology

Histological evidence of inflammation in routine PD patients membranes is limited with the largest peritoneal biopsy study (18) finding only 12% of patients with inflammatory changes (75% of which were chronic inflammation). Whilst there is no real evidence of overt inflammation, there is a little evidence for an increase in subtle inflammatory changes with uraemia and PD. Kihm et al found an increase in IL-6 and NF-κB staining but no increase in CD3 staining in uraemic patients, whilst 4 PD patients with before and after biopsies had an increase in CD3 staining. (89) Milky spots have also been shown to increase in either number or size in both animal and human studies**.** (90) Few other studies have looked for subtle changes.

One of the strongest pieces of evidence for an association between inflammation and PSTR comes from study of 42 uraemic patients who had a peritoneal biopsy at the point of catheter insertion. The concentration of peritoneal macrophages correlated very strongly (r=0.61) with the PSTR measured within 6 months of starting PD. (91)

2.3.2.2 Cellular profile

Macrophages, lymphocytes and neutrophils have all been detected in dialysate (92) with macrophages up to 70% of all the leucocytes (93) although there are wide variations between individuals**.** (94) Mast cells are found within the peritoneum and increase with PD and peritonitis**.** Mast cell deficient rats had less peritoneal cell influx and omental changes, but no change in functional parameters or angiogenesis, fibrosis or mesothelial cell damage**.** (95) The intra-peritoneal lymphocyte population consists of 10-20% B lymphocytes with the remainder T cells, (94) which are mostly composed of effector memory T cells, probably resident within the peritoneum**.** (96)

Endothelial cells play an active role in inflammation and a local increase in rolling, adhesion and extravasation of leucocytes has been demonstrated in animal PD models. Mesothelial cells usual function is to provide a frictionless and protective layer, with this latter role responsible for the secretion of chemokines including IL-8 or MCP-1, as well as inflammatory cytokines like IL-6, in response to stimuli including AGE's, GDPs, peritonitis and glucose. (97) There is also constitutive expression of Toll-like receptors (TLR) -1, 2, 3, 4, 5 and 6 by mesothelial cells.

2.3.2.3 Cytokine profile

Studies of cytokines are frequently hard to interpret for a number of reasons. First, as cytokines are usually small enough to diffuse across the peritoneal membrane, and would also arrive intra-peritoneally by convection, intra-peritoneal levels may reflect this, rather than local production. Second, there is no 'normal' control group against whom comparisons can be made when examining dialysate, as dialysate by definition is not 'normal'. Third, the concentrations of cytokines do not necessarily reflect activity as this is complicated by the presence of natural inhibitors e.g. IL-1β with IL-1RA, and the presence of cytokines with antagonistic activity e.g. pro-inflammatory TNF-α with anti-inflammatory IL-10. Fourth, cytokine concentrations will be dependent on the dwell length so appearance rates must be calculated to take this into account. Fifth, some established immunoassays may not differentiate between active molecules and those bound to their specific inhibitor. Finally, the functional significance of differences in concentrations is often unclear as less inflammatory cytokine production may represent less inflammation and damage, but may also represent impaired cellular function and therefore defense against micro-organisms.

IL-6 is a pleiotropic cytokine secreted by many cell types including T cells, macrophages, adipocytes, myocytes, vascular smooth muscle cells and osteoblasts with effects including immunoglobulin production, osteoclast activation, platelet production, fever, myeloma and mesangial cell proliferation, and the acute phase response by hepatocytes amongst many others. IL-6 acts through a signaling complex of IL-6 receptor (IL-6R), for which expression is limited to a few cell types, and

ubiquitously expressed gp130, but IL-6R can be alternatively spliced or shed to create soluble IL-6R. When bound to IL-6, soluble IL-6R, through gp130 (trans-signaling), has a different effect thought to mediate the transition from acute to chronic inflammation. (98)

IL-6 is routinely detectable at higher concentrations in the dialysate than in the serum, implying local production, and there is significant correlation between dialysate IL-6 and solute transport**,** (99,100) including in multivariable modeling**,** (101) although how this correlates with inflammation and how much of this relationship could be due to filtration of plasma IL-6 remains unclear.

Dialysate IL-1β, TNF-α, IFN-γ and IL-10 levels are usually low to undetectable, all rising sharply with peritonitis**,** (102–105) but in PD patients IL-8 has an elevated serum level and an approximately equivalent dialysate concentration IL-8 local production usually. (106,107) MCP-1 and VEGF are also usually detectable in dialysate**.** (101)

2.3.3 Systemic inflammation

2.3.3.1 Is systemic inflammation important in PD patients?

As discussed below, there is a well described association of muscle wasting and cardiovascular disease with inflammation in ESRF patients, which has been described as the 'MIA' (malnutrition, inflammation, atherosclerosis) syndrome (108) and all 3 parts of this syndrome are associated with an increased mortality.

2.3.3.1.1 Inflammation and cardiovascular disease

Inflammation is now recognised as playing a role in CVD within the normal population (109) thought to occur through an endothelial effect**,** and inflammatory markers, particularly IL-6, are significantly associated with mortality in ESRF patients, (110) a group that have a high risk of CVD. Studies have subsequently confirmed the link within ESRF patients between inflammatory markers and cardiovascular death, (111) but CVD in ESRF is histologically different from classical occlusive atherosclerotic disease, being characterised by calcification of the arterial media, rather than the

intima.(112) Inflammatory markers in ESRF patients have been associated with typical atherosclerotic disease (113) but there are an increasing number of studies demonstrating a link with arterial calcification. (114–116)

Fetuin-A is a negative acute phase protein that is also a calcification inhibitor and low levels of this are associated with malnutrition, atherosclerosis, inflammation and mortality in dialysis patients. (117) Osteoprotegerin (OPG), a member of the TNF-receptor superfamily, is an inhibitor of RANKL (Receptor activator of NF-κB Ligand), a member of the TNF superfamily which induces osteoclast differentiation and maturation, and elevated levels of OPG are associated with both CRP and calcification,(118) suggesting a protective response secondary to inflammation and calcification.

2.3.3.1.2 Inflammation and body composition

Muscle wasting is recognised in many inflammatory conditions and it has now been suggested that subclinical inflammation may be partially responsible even for wasting associated with aging. (119) IL-6 promotes muscle protein catabolism,(120) and cancer associated cachexia,(121) whilst high levels are associated with anorexia in dialysis patients. (122)

TNF-α is also associated with cachexia,(123) with a variety of possible mechanisms including activation of the ubiquitin-proteasome system,(124) inhibition of MyoD transcription (125) and through anorexia.

The functional significance of this interaction between inflammation and catabolism is illustrated by a study demonstrating that polymorphisms predicting high levels of TNF- α and IL-6 were all independently associated with higher comorbidity and lower functional status and low IL-10 producers predicted lower functional status (closely associated with muscle function and composition) in haemodialysis patients. (126)

2.3.4 Fibrosis

As discussed above, there is clear evidence of an increase in peritoneal fibrosis in uraemic patients which progresses dramatically with time on PD. The drivers for this must ultimately be the same as for inflammation i.e. uraemia, the presence of a catheter, peritonitis and bioincompatibility of dialysate. Which of these is most important is unclear due to the difficulties in collecting biopsies from patients, or in using indirect evidence such as the osmotic conductance to glucose, or trying to separate the effect of fibrosis on UF capacity from the other factors affecting this. Studies have focused on determining molecular mediators instead.

2.3.4.1 Advanced glycation end products

Advanced glycation end (AGE's) products are irreversibly altered proteins including pentosidine and carboxymethyllysine, that are associated with uraemia, and one of the most potent inducers of AGE's are the glucose degradation products (GDPs) such as methylglyoxal and 3-deoxyglucosone found in standard dialysate.(127) GDPs alter the structure and function of mesothelial cells (128) and AGE's induce collagen synthesis through TGF-β**,**(129) accumulate in the peritoneal vasculature and interstitium**,** (130)and correlate with fibrosis, solute transport and ultrafiltration dysfunction**.**(131)

2.3.4.2 Inflammation

Inflammation has long been recognised as the precursor to the healing process, of which fibrosis is a necessary part, but there is now evidence that inflammation can both exacerbate and aid in the resolution of fibrosis**.**(132) Within the peritoneum there are several studies suggesting a link between inflammation and subsequent fibrosis but the complexity is demonstrated in a study of the effects of IL-1β or TNF-α expression where the subsequent fibrosis resolved by 21 days when induced by TNF-α but IL-1β induced fibrosis persisted, in association with greater inflammation and higher levels of TGF-β and TIMP-1.(133) Peritonitis has been studied clinically and found to upregulate IL-1β, IL-6, TGF-β and bFGF sharply on the first day but levels remained elevated for at least 6 weeks after, consistent with longer term effects upon fibrosis.(134)

2.3.4.3 Renin-angiotensin-aldosterone system

As there is for renal fibrosis and cardiac hypertrophy, there is evidence of a pro-fibrotic role for the renin-angiotensin-aldosterone system (RAAS) within the peritoneum,. Components of the RAAS are expressed within mesothelial cells, and are upregulated in the presence of inflammation and exposure to peritoneal dialysate. The high glucose concentration, low pH, and the presence of GDPs in dialysate have all been implicated in modulation of this system.(135,136) Furthermore, activation of the RAAS, as well as the downstream production of transforming growth factor-beta, contributes to epithelial-to-mesenchymal transformation of mesothelial cells and to increased VEGF production. Several animal models have shown that fibrosis can be ameliorated by blocking the RAAS.(137)

2.3.4.4 Transforming growth factor-β

TGF-β has a central role in fibrosis in a wide variety of disease types and this was also suggested in peritoneal fibrosis by using adeno-virally mediated gene transfer in a rat model.(138) TGF-β has a wide variety of effects such as fibroblast activation, collagen deposition, inhibition of fibrinolysis through PAI-1, maintenance of fibrosis through inhibition of matrix metalloproteinases, angiogenesis, EMT and immunomodulatory effects including inhibition of T helper cell proliferation and increased Th17 and regulatory T cell production. Dialysate levels of TGF-β were higher than predicted by diffusion from serum,(139) and TGF-β1 mRNA was detectable in dialysate macrophages, suggesting local production. There have been conflicting results surrounding the association between dialysate levels of TGF-β and solute transport (100,139,140) although when TGF-β is expressed by adeno-virus vector in an animal model, there is an increase in solute transport.(138) Dialysate levels are particularly difficult to interpret for TGF-β as it is secreted as a complex with Latency –Associated Peptide and one of the Latent TGF Binding Proteins in an inactive form so it is unclear what the levels represent.

2.3.4.5 Other mediators

Other putative mediators of fibrosis include FGF-2, connective tissue growth factor (CTGF), TGF-β's 2 and 3, platelet derived growth factor (PDGF) and PAI-1. Molecules such as the MMP's may also have

a role, partly through their role as collagenases and their balance with TIMP's, but also in the induction of EMT (MMP-2) although this latter factor has mostly been studied within the kidney.

2.3.5 Blood and lymph vessel changes

The type of angiogenesis of relevance to peritoneal dialysis is sprouting angiogenesis, initiated by growth factors that stimulate the endothelial cells to degrade the basement membrane through protease production. Endothelial cells then proliferate to form sprouts and migrate through the use of integrins towards the angiogenic stimulus. Some of the main signaling molecules involved include the VEGF family, fibroblast growth factors (particularly 1 and 2), angiopoietins, ephrins, platelet derived growth factors, transforming growth factor-β and endothelial nitric oxide synthase.

2.3.5.1 Angiogenesis and solute transport

Angiogenesis is well established as occurring in experimental PD, and correlates with solute transport but using solute transport as a direct marker for angiogenesis does have some flaws as solute transport actually represents perfused capillaries in contact with dialysate, so could be influenced by vasodilation. Some direct studies of causation of peritoneal angiogenesis have been done and they mostly suggest that the same stimuli for faster solute transport induce angiogenesis. For instance, activation of the receptor for advanced glycation end products (RAGE) induces VEGF and angiogenesis**,**(141) glucose degradation products (GDPs) induce mesothelial production of VEGF (142) whilst a GDP scavenger (aminoguanidine) reduced angiogenesis (143) and dialysate lactate increase angiogenesis**.**(144) EMT associated with more VEGF production and faster solute transport**.**(82) The direct relevance to solute transport is demonstrated by the fact that VEGF polymorphisms predicted a greater increase in solute transport at 1 year follow up of incident patients**.**(39)

2.3.5.2 Angiogenesis and the link with inflammation

Inflammation has a recognised association with angiogenesis, particularly from cancer research where inflammation is thought to adversely affect outcomes through blood vessel formation and

subsequent tumour growth. Of particular relevance to peritoneal dialysis is the fact that in a variety of situations IL-6 induces VEGF,(145) although IL-6 requires sIL-6R and angiogenesis is completely inhibited by VEGF inhibitors (146) or tocilizumab.(147) IL-1β and α, TNF-α, oncostatin M and IL-8 also induce VEGF. Specifically in PD, the linkage has been demonstrated in animal models where both IL-1 and TNF-a administered intraperitoneally induced VEGF and angiogenesis in rats (133) and where LPS induced an increase in VEGF and solute transport.(148) Angiogenesis also develops in tandem with fibrosis,(149) and interventions to reduce angiogenesis also decrease fibrosis.(150)

Conversely, angiogenesis can affect inflammation – Placental GF induced IL-6 and TNF-α by mononuclear cells,(151) transgenic PlGF induced dermal inflammation (152) VEGF can increase TNF-α and IL-6 production by peripheral blood mononuclear cells (153) and angiopoietin-2 may have a role in the widespread inflammation in critically ill patients, including prognostic significance.(154) Of relevance to PD is the observation that angiopoietin-2 deficient mice cannot mount an inflammatory response to Staphylococcus aureus peritonitis.(155) Together, these findings illustrate the difficulty of investigating inflammation, angiogenesis and fibrosis in isolation, given their interconnected nature.

2.3.5.3 Lymphangiogenesis

There has been a large increase recently in the investigation of lymphangiogenesis due to the identification of lymphatic vessel specific markers, which has started to allow the identification of some of the molecular mechanisms controlling this process. A central role has been established for VEGF-C through VEGFR-3 (156) as well as roles for VEGF-A and D and angiopoietin-2.(157) There is an association with inflammation, with evidence of lymphangiogenesis induction by TNFα,(158) Toll-like receptor 4/Lipopolysaccharide,(159) and increased lymphangiogenesis in inflammatory joints.(160) TGF-β appears to inhibit lymphangiogenesis.(161,162) There is therefore good reason to suspect abnormalities in lymphangiogenesis will exist in peritoneal dialysis but there has been only one animal study (163) directly investigating lymphangiogenesis (in relation to celecoxib use) and

one study of VEGF-C levels in dialysate where they are related to faster solute transport and less UF.(164) Despite the prominence of oedema in renal failure, it is not known if there is any uraemic effect upon the regulation of lymphangiogenesis.

2.3.6 Is there a link with Encapsulating Peritoneal Sclerosis?

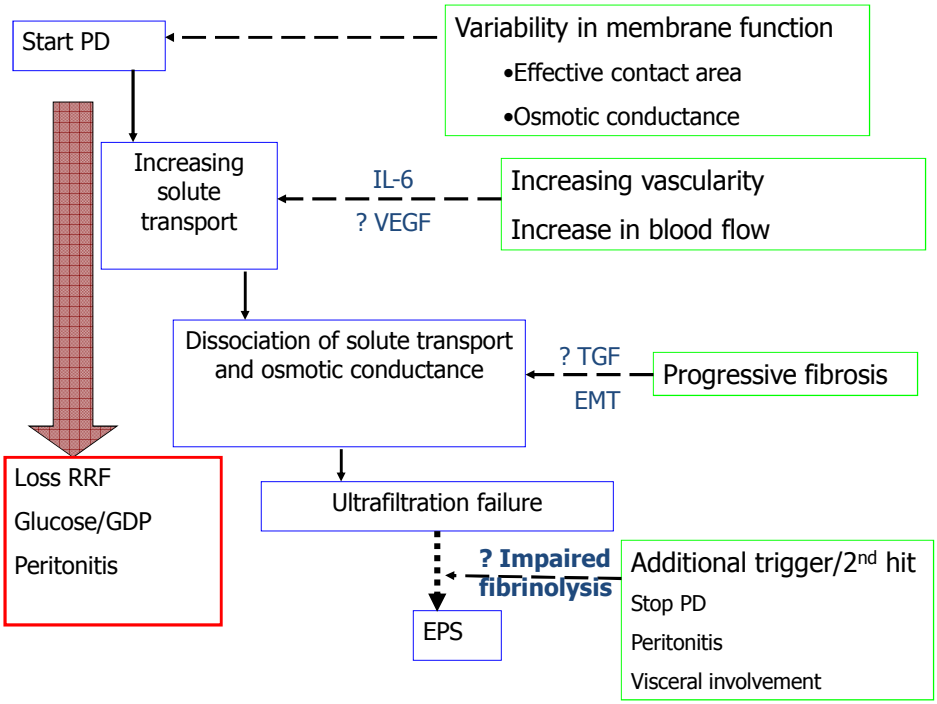
Encapsulating peritoneal sclerosis is an uncommon condition primarily occurring in patients with prolonged exposure to PD, where a cocoon forms around the intestine causing obstruction, malnutrition and a high mortality. Histological changes consist of increased membrane fibrosis, particularly in visceral peritoneum, inflammation, and vascular occlusion and calcification. This has been compared with patients with 'simple sclerosis' where, as with the Peritoneal Biopsy Registry, parietal fibrosis is more significant.(165)

Studies to date have identified a fast solute transport with a low ultrafiltration capacity at the time of stopping PD in those patients who develop EPS.(166) As time on PD is associated with increased fibrosis and impaired ultrafiltration capacity, there is therefore circumstantial reason to suspect that a gradual increase in membrane fibrosis is a significant risk factor for EPS although it still remains unclear why certain patients with fibrosis would subsequently develop EPS whilst others do not. Possible 'second hits' include stopping PD, peritonitis or visceral involvement.

2.4 Conclusion

At the start of PD there is variability in solute transport which remains mostly unexplained. During PD, the peritoneum undergoes histological changes characterised primarily by angiogenesis and fibrosis with the functional consequences of fast solute transport and impaired ultrafiltration capacity leading to increased mortality. The drivers of this change are peritonitis, loss of residual renal function and subsequent greater bioincompatible fluid exposure. The only molecule consistently linked with these processes is intra-peritoneal IL-6 with solute transport but the prognostic and diagnostic information this adds is unclear. The role of inflammation, whether systemic or local, is also unclear. The final result of these changes, if left unrecognised and unmanaged, is likely to be a high risk of EPS. A summary of this model is displayed below (Figure 2-5). The purpose of this thesis is to investigate the roles that local and systemic inflammation are playing, whilst seeking to establish clinically useful biomarkers.

Figure 2-5: Pattern of Cumulative Membrane Damage During Peritoneal Dialysis



3 Methods and Materials

3.1 Studies

3.1.1 Stoke PD study

This is a single centre, prospective, cohort study commenced in 1990 by Professor Simon Davies. All patients commencing PD in University Hospital of North Staffordshire were included and demography recorded at this point. Routinely collected clinical data was also recorded 6 monthly, including blood tests, PET's (D/P Cr and UF capacity), adequacy measurements (both renal and dialysis) and PD prescription including dialysate glucose exposure, as well as peritonitis events when they occurred. The outcome at the end of PD and the eventual date of death were also recorded. The Stoke comorbidity score was validated in this study (167) and this was subsequently available for all patients.

The database started as a spreadsheet but iteratively evolved into a bespoke Access database (PDDB) which is used as both a clinical and research tool (see Section 3.2.1 below for details). The data has been validated through numerous previous studies on longitudinal change in peritoneal function, and its relationship to mortality, peritonitis and dialysate glucose exposure.(17,32,42,56) At the time of use in this thesis, there were 692 patients included.

3.1.2 GLOBAL Fluid Study

This is a multi-centre, multi-national, prospective, cohort study commenced in 2002 by Professors Simon Davies and Nick Topley and funded by Baxter Healthcare. The same clinical and demographic data as in the Stoke PD study was collected, including the Stoke comorbidity score, but a dialysate and plasma sample was also taken when clinical samples were being acquired (i.e. not during fasting conditions). As the study was a pragmatic, large study with a limited budget the timing of sample acquisition was not strictly mandated but occurred during either PET or occasionally adequacy testing.

The regularity of the data acquisition was allowed to vary according to the local centres practice pattern (e.g. in Stoke, data and samples were collected every 6 months). 16 centres from 6 countries recruited patients (see table Table 3-1**)**

Table 3-1: List of GLOBAL Fluid Study Centres

Country	Centre	Included in initial analysis	Patient Numbers
UK	University Hospital of North Staffordshire, Stoke-on-Trent	Yes	209
	Ipswich Hospital, Ipswich	Yes	25
	Addenbrooke's Hospital, Cambridge	Yes	27
	Morriston Hospital, Swansea	Yes	118
	Manchester Royal Infirmary, Manchester	Yes	116
	University Hospital of Wales, Cardiff	No	178
	Queen Elizabeth Hospital, Birmingham	No	9
Canada	Dr. Georges L.Dumont Hospital, Moncton	Yes	41
	Edmonton General Hospital, Edmonton	Yes	70
Korea	Yeungnam University Hospital, Daegu	Yes	55
	Soon Chun Hyang University, Seoul	Yes	53 included (30 excluded due to missing batch of samples)
	Kyungpook National University Hospital, Daegu	Yes	245
Hong Kong	Princess Margaret Hospital, Kowloon	No	4
Israel	Carmel Hospital, Haifa	No	18
	Assaf Harofeh Hospital, Zrifin	No	18
Belgium	University Hopsital of Ghent, Ghent	No	20

The dialysate and plasma samples were kept locally then transferred in batches for storage at the central laboratory at Cardiff University School of Medicine, with all storage at -80^{0}C. Clinical data on CRF's was also sent there for input onto the Global Database, unless the units used PDDB.

3.1.3 AnzData

AnzData is a registry funded by Kidney Health Australia, the New Zealand government and the Commonwealth through the Organ and Tissue Authority which collects a limited dataset on all dialysis and transplant patients throughout Australia and New Zealand. Data is collected by a web based data form as events occur and by an annual paper-based survey, with data held at the Royal Adelaide Hospital. The data has been collected since dialysis commenced in these countries and has been extensively validated, with 22 publications in 2012 alone.

Data is collected on basic demography, comorbidity and dialysis details. Comorbidity is collected through specific questions for chronic lung disease, coronary artery disease, peripheral vascular disease and cerebrovascular disease, diabetes and smoking, as well as a free text box to enter other comorbidities. PD specific data collected includes the initial PET D/P Cr result, adequacy results for dialysate and residual renal function and type of PD solutions used as well as a specific form to report instances of peritonitis. There is also a data dictionary specifying causes of dialysis modality switching and causes of death that are recorded.

3.1.4 Scottish Renal Registry

Data from Scottish renal units are available from 1960 with a small data set which was initially sent to the European Renal Association- European Dialysis Transplant Association (ERA-EDTA) registry. In 1991 a computer based registry was established for patients receiving RRT for ESRD in Scotland, funded by the National Health Service in Scotland.

Data collection is through a paper based form, with routine collection of demography and dialysis details but not comorbidity. PD details include peritonitis details and dialysate adequacy. EPS data was collected for a specific project (168) where all 10 renal units were contacted and asked to identify all EPS patients and the diagnosis was then independently validated by the study team. The data has been extensively validated by the Registry's internal processes, with numerous publications resulting.

3.1.5 PD-CRAFT

Data from this study was not used directly in this thesis but will be briefly described as it underpins the strategy described in Chapter 8, as well as providing some of the opportunities for further work described in Section 10.4.

The primary aim of this National Institute for Health Research Research for Patient Benefit grant funded study is to validate a prognostic model for EPS, which is being developed with AnzData and SRR data. It is an observational cohort study, with all UK patients on PD eligible for inclusion but with a focus on prevalent PD patients. Standard demographic and clinical data, including peritoneal membrane function testing where it is routinely performed, will be collected and patients will be followed up until study end or death. Currently, 43 UK renal centres are recruiting and the target patient number is 1,600 with over 700 recruited to date.

An ancillary study, funded by the Baxter Extra-mural Grant Programme, is collecting dialysate and plasma samples from 300 long term PD patients, providing a resource to check candidate biomarkers as predictors of EPS. A further ancillary study is collecting data to examine markers associated with glucose metabolism as well as abdominal circumference.

3.2 Data Validation and Integrity

3.2.1 Database design

The study database (Peritoneal Dialysis DataBase, PDDB) is a bespoke database which evolved over time, building on the GFS database and the Stoke PD study database, which is designed specifically for clinical PD studies. A Microsoft Access database was originally developed by Dr Kieron Donovan[1] from Cardiff for the GFS, and functionality was added by Dr James Chess[2] for the Swansea Prevalent

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[2] Consultant Nephrologist, Abertawe Bro Morgannwg University Health Board, Swansea

Study. This structure was then significantly reworked by Dr Christopher Huckvale[3], incorporating a lot of the structure that Prof Simon Davies had developed for the research database for the Stoke PD study, into what is now PDDB. A PDDB copy is used for the ongoing Stoke PD study, with separate copies used for the Global Fluid Study (see study descriptions for description of data in these studies).

PDDB has itself has evolved iteratively, with the latest version being 4.16 and now incorporating the functions in Table 3-2.

Table 3-2: PDDB Functions

Data type/range constraints
Internal data consistency checks
Extraction of anonymised encrypted consented data only
Patient level graphical timeline display of data
Automatic Backup

3.2.2 Data entry and checking procedure

Data was managed in different ways depending on the centre involved. For most centres data was recorded on paper based Case Report Forms, then transferred to Cardiff for entry into the study database, but for Stoke data was entered directly into PDDB as it is used for clinical management. For Ipswich, Manchester and Cambridge data was initially entered in Cardiff via CRF but subsequently a centre-specific version of the data was created and this was used for direct data entry at the centres. For the final analysis, all centre-specific versions were combined into an analysis spreadsheet.

[3] Clinical Research Fellow, Dept of Primary Care and Public Health, University College London

Table 3-3: Data Checking Processes

Validation on entry to database
Database allowed ranges
PDDB automatic checks
Missing Data Check
Data Values Check
Data Sense Checking

Data checking occurred at several stages as shown in Table 3-3. The missing data check, the data values check and the data sense checking were performed on centre-specific versions using Structured Query Language (SQL), and issues resolved with the individual units if possible. The data sense checking rules are illustrated in 11.4.

3.3 Measurements

3.3.1 Inflammatory Markers

Enzyme-Linked Immuno-Sorbent Assays (ELISA) are a standard laboratory method used to detect a very wide range of antigens. An antibody specific to the antigen of interest is bound to a plate or well and the solution of interest is applied. The solution is washed off and a second 'detection' antibody is applied. An antibody to the detection antibody is then applied, but it is conjugated to an enzyme that will generate a signal.

Electrochemiluminescence (ECL) is a modification of this process where an electrically generated charge triggers a chemical reaction that leads to luminescence. It has the benefit of using small volumes, rapidity and being suitable for use in a multi-array plate. It is also sensitive as the trigger (electrical) is separate from the detection method (luminescence).

The technique used for analysis of the GFS samples (Sections 4, 5 and 6) was ECL, using a Multi-Array 4-spot inflammatory plex from Meso-Scale Discovery (MSD). This assays for human interleukin-1β

(IL-1β), tumour necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and interleukin-6 (IL-6) and they were run by Miss Ann Kift-Morgan and Mrs Charlotte James at Central Biotechnology Services, Cardiff University.

3.3.2 Peritoneal Solute Transport Rate

In all studies involved in this thesis, the method used for measuring PSTR was the D/P Cr derived from the 4 hour PET. All measurements were performed locally, and any correction for dialysate glucose concentrations was locally determined.

3.3.3 Ultrafiltration Capacity

As in the case of PSTR, all measurements of UF capacity were based on the 4 hour PET although the glucose concentration used was either 2.27% or 3.86%. Local corrections for bag overfilling were applied.

3.4 Statistical Models

3.4.1 Linear regression

The basic technique of simple linear regression is to model the relationship between a response variable, y, and a predictor variable, x, using linear coefficients with values estimated from the dataset. An example of the use of this would be to model the relationship between PSTR and dialysate IL-6 levels, allowing quantification of the relationship and a test of whether this relationship is stronger than is likely to have occurred by chance.

Equation 3-1: Simple Linear Regression

$$y_i = \alpha + \beta x_i + \varepsilon_i$$

Where y = response variable, x = predictor variable, i = individual within the dataset and ε = unexplained variation, or residual.

3.4.1.1 Multivariable linear regression

Simple linear regression with one predictor variable is easily extended to multiple variables. Using the example of regressing dialysate IL-6 levels on PSTR, this could be extended to regressing a variety of other variables simultaneously. If the dialysate IL-6 was significantly associated on univariable regression but was no longer significant on multivariable testing, this would imply that the univariable association was most likely due to dialysate IL-6 correlating with another variable that had a 'true' underlying relationship with PSTR.

Equation 3-2: Multivariable Linear Regression

$$y_i = \alpha + \sum_{k=1}^{n} \beta_k x_{k,i} + \varepsilon_i$$

where there are n predictor variables. There are some important assumptions for these models. Firstly, observations have to be independent of each other e.g. if repeated observations are taken from one subject, they will be more closely correlated than others and therefore not independent. Deviation from this assumption results in correlated residuals. Secondly, for the most commonly used forms of this model, the residuals must be normally distributed which typically involves the dependent variable being normally distributed. Thirdly, homoscedasticity should hold, where the residuals have a constant variance plotted against the predicted dependent variables. Fourthly, the predictor variables should not demonstrate multicollinearity. This occurs when the predictor variables are highly correlated, causing the estimated variance in the predictor coefficient to be inflated.

3.4.1.2 Analysis of clustered data

If the dataset to be analysed contains data that is clustered, as is the case for chapters 4, 6, 7 and 9, this breaks the assumption of independence of errors. Clustered data commonly occurs when repeated measures are taken from one person, or if data on multiple individuals from several centres are collected. Data from different hospitals is one such source as different hospitals tend to serve populations that differ in many ways that are both measurable (e.g. ethnicity, socioeconomic deprivation) and unmeasured (e.g. genetic differences, unknown environmental factors). To assess

level of variance accounted for by clustering, the intra-cluster correlation coefficient (ICC) is used. If a model is developed

Equation 3-3: Simple Random Effects Model

$$y_{ij} = \alpha + \mu_j + \varepsilon_{ij}$$

for i level 1 units (or patients) and j level 2 units (or hospitals) such that y_{ij} represents the i'th subject from the j'th centre, with μ = level 2 residual and ε = level 1 residual then the variance is partitioned into σ_μ^2 and σ_ε^2. The ICC is then defined as

Equation 3-4: Intra-cluster Correlation Coefficient

$$ICC = \frac{\sigma_\mu^2}{(\sigma_\mu^2 + \sigma_\varepsilon^2)}$$

If the ICC is significant then estimates from standard linear regression will be biased and the standard error will be misleadingly low with an increased chance of a type I error. To get around this, multilevel models have been developed:

Equation 3-5: Multilevel Linear Regression

$$y_{ij} = \alpha + \sum_{k=1}^{n} \beta_k x_{k,ij} + \mu_j + \varepsilon_{ij}$$

for n covariates. This is a basic form with only a random intercept (intercept = $\alpha + \mu_j$) but it can be adapted to include random slopes by replacing β_k with ($\beta_k + \gamma_j$) where γ_j is a random effects component varying between level 2 units. More levels can be created to allow, for example, grouping by country, and hospital and patient. When the residuals are normally distributed, these models are also referred to as linear mixed models, but they can incorporate continuous non-normal, binary and categorical responses in generalised linear mixed models. These models were used to analyse:

- determinants of dialysate and plasma IL-6 and PSTR (chapter 4)

- determinants of inflammatory cytokines and PSTR to establish whether they differed between patients with subsequent EPS and those without (chapter 6)
- whether dialysate glucose load independently predicted random blood glucose levels (chapter 9)

Other techniques are available to analyse clustered data. These include generalised estimating equations which, although more robust to misspecification, provides population-averaged coefficients, does not allow for analysis of the different levels of variation and requires careful handling of missing data. Repeated-measures analysis of variance has more restrictive assumptions including the same number of measures per cluster and sphericity, where there is constant variance and covariance.

3.4.2 Survival Analysis

3.4.2.1 Survival and hazard functions

Longitudinal studies often measure events that may occur during follow up, most commonly death in the medical literature, and they require a particular analytic approach, as is the case for analyses in chapters 4, 8 and 9. A key principle for these survival analyses is the survival function, S, defined as the probability of the event of interest occurring after a given time:

Equation 3-6: Survival Function

$$S(t) = \Pr(T > t)$$

where t is time, Pr = probability and T is a random variable denoting time of death. A Kaplan-Meier plot provides an estimate of this function. The cumulative incidence function of T, (F(t)), is given by

Equation 3-7: Cumulative Incidence Function

$$CIF(t) = 1 - S(t)$$

A further concept is the hazard function, λ, which represents the event rate at a given time:

Equation 3-8: Hazard Function

$$\lambda(t) = \lim_{dt \to 0} \frac{\Pr(t \leq T < t + dt \mid T \geq t)}{dt}$$

This is linked to the survival function through the cumulative hazard function, Λ, defined as the integral of the hazard function and therefore the accumulated hazard over time but which has no upper bound:

Equation 3-9: Cumulative Hazard Function

$$\Lambda(t) = \int_0^t \lambda(u)du = -\log S(t)$$

Censoring, where follow up is stopped before the event of interest (right censoring), is common in survival studies and any analysis must take this into account.

3.4.2.2 Cox models

One of the most widely used models is the Cox proportional hazards model, as this is a semiparametric model where the baseline hazard function is not specified: Multivariable Cox models were used to examine the relationships between patient survival and: inflammatory cytokines (chapter 4), variables available for a prognostic model of EPS (chapter 8) and random blood glucose levels (chapter 9).

Equation 3-10: Cox Model

$$\lambda(t|x) = \lambda_0(t)e^{(\beta_1 x_{i1} + \cdots + \beta_k x_{ik})}$$

Results from the Cox model are typically given as a hazard ratio (i.e. the exponentiated value of the coefficient β). The Cox model also has the advantage of being able to incorporate time varying effects, whereby a predictor value can change over time, or the effect of the predictor can change. It is also possible to adjust for an effect without having to estimate the effect by performing a stratified analysis where the strata are incorporated into the baseline hazard.

The assumptions of the Cox model are: non-informative censoring (informative censoring occurring when cases are censored by a non-random method), and proportional hazards, whereby the effect of a unit change in predictor variable remains constant over time. The former issue is avoided by study design and the latter issue is tested for by a variety of methods, such as log-log plots or regressing Schoenfeld residuals on time.

3.4.2.3 Competing Risks

Cox models perform very well for single outcomes such as death but there are many situations in medical studies where more than one mutually exclusive outcomes are possible, as found in chapter 8. If any of these multiple outcomes is non-random, then a Cox model of another outcome has the non-informative censoring assumption violated. Furthermore, if predictor variables associate with more than one outcome then a Cox model cannot provide estimates of the probability for an individual of experiencing an event, as this will be affected by the effect of the other events. One consequence of applying simple survival analysis techniques is that the Kaplan-Meier estimator of the survival function will overestimate the risk of an event.

A common situation would be a study of an event other than death and a standard survival analysis would treat death as a censoring event and those subjects who die would be treated as remaining at risk of a further event. If age affected the event of interest, then the effect on the incidence of the event would also depend on the incidence of death but this would not be apparent from a Cox model. This is a particular concern for EPS as this is strongly associated with a prolonged duration of PD, but patients with a high risk of death are unlikely to survive for long enough to be at a significant risk of EPS.

A better alternative is to use a competing risks (CR) analysis, although it is recommended that this is done in conjunction with a Cox model of a cause-specific hazard to aid interpretation. For a CR analysis, use is made of the cumulative incidence function (CIF) providing the probability of an event having occurred by a given time point, rather than the survival function referred to above as the

interpretation of S is more complicated in the CR scenario. The CIF for cause z, with a survival function S defined as survival free from any event, covariates represented by X and a cause specific hazard λ is defined as:

Equation 3-11: Cumulative Incidence Function in Competing Risks

$$CIF_z(t) = \int_0^t \lambda_z(u|X)S(u)du$$

This explicitly states that the risk of any event is dependent on other events not occurring. The sum of the CIF's for all events are then equal to 1 – S(t).

As can be seen from the equations above, for a Cox model the CIF can, with mathematical transformations, be linked directly to the hazard, but for a competing risks scenario, the CIF now depends on other events too so the effect of a covariate on the CIF is no longer clear. Because of this Fine and Gray (169) developed the subdistribution hazard, h, which links a covariate directly to the CIF:

Equation 3-12: Subdistribution Hazard

$$h_1(t) = \lim_{dt\to 0} \frac{\Pr(t \leq T < t + dt, z = 1|T \geq t \cup (T \leq t \cap z \neq 1)}{dt}$$

Equation 3-13: Subdistribution Hazard and Cumulative Incidence Function

$$h_1(t|x) = -\frac{\log\{1 - CIF_1(t|x)\}}{dt}$$

This models the effect of a covariate upon the overall risk of an event, taking into account the effect upon competing events. The CR model uses this in a very similar method to the Cox model:

Equation 3-14: Competing Risks Model

$$h_1(t|x) = h_{1,0}(t)e^{(\beta_1 x_{i1} + \cdots + \beta_k x_{ik})}$$

In this model, the proportionality assumption still holds, the baseline subhazard does not require specification and the results are reported as subdistribution hazard ratios.

Because of the benefits of a competing risks model, this was used to establish independent predictors of EPS taking into account the risk of death as the first step to establishing a prognostic model for EPS (chapter 8).

3.4.3 Fractional Polynomials

One of the problems with normal linear regression is that relationships are assumed to take particular forms. Usually this will be a simple straight line relationship as demonstrated in Equation 3-1 and Figure 3-1, but if the true relationship between them assumes a different form, as in Figure 3-2, a linear relationship is at best inaccurate. The possibility that PSTR follows a non-linear path with time is explored in chapter 5.

Figure 3-1: Linear Relationship Between 2 variables

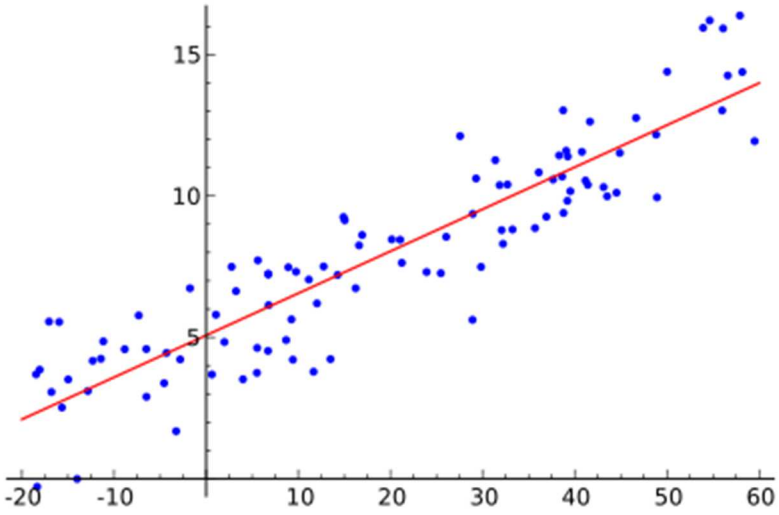
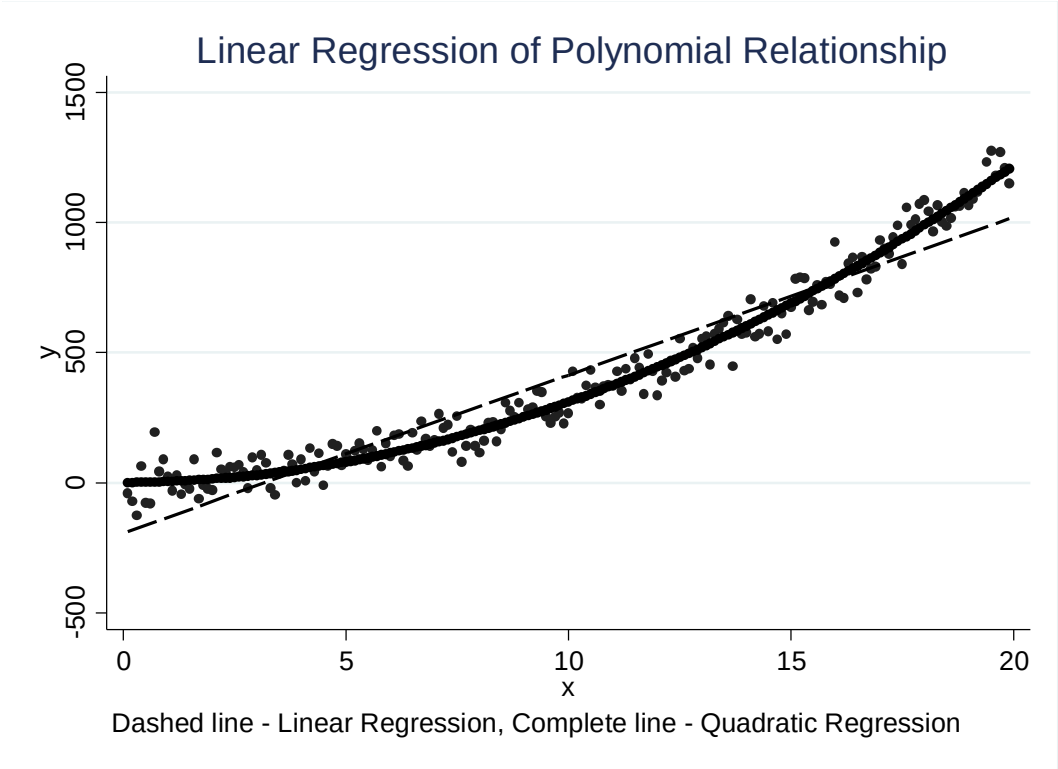


Figure 3-2: Linear Regression of Polynomial Relationship



With standard regression if a non-linear relationship is suspected, quadratic terms can be applied but there is a practical limit on the number of different forms that can be tried. To achieve a regression fit without assumptions being made about the shape of the true fit, fractional polynomial regression tries different combinations of different transformations of the predictor variable to achieve the best fit of the response variable. An example of the regression equations tried is shown in Equation 3-15.

Equation 3-15: Example Fractional Polynomial Equation

$$y = \beta_1 x_1^{-1} + \beta_2 x_1^3 + \beta_3 x_1^3 \log(x_1)$$

For each equation tried, a measure of the fit of the model (deviance = -2*log likelihood) is calculated and the best model is used. The transformations tried are from the ladder of powers and typically a limited range is necessary to provide a very wide range of potential fits e.g. {-2, -1, -0.5, 0, 0.5, 1, 2, 3} where 0 = logarithmic transformation. The number of terms in the model is variable but again,

typically only 3 or 4 provide a very wide range of fits. Other options that do not assume specific forms exist, primarily including locally weighted scatterplot smoothing (lowess) regression but this does not provide a regression function easily represented by a mathematical function and is therefore less easily reproduced.

3.4.4 Missing Data

As with all studies, the issue of missing data had to be considered in all of the analyses. There are generally considered to be 3 types of missing data, as shown in Table 3-4.

Table 3-4: Types of Missing Data

Missing Completely At Random (MCAR)
Missing At Random (MAR)
Missing Not At Random MNAR)

Missing Completely At Random (MCAR) comprises data missing by a random method unrelated to the observed or missing data, such as a sample being lost or smashed. A wide range of analyses remain valid in this situation.

If data is Missing At Random (MAR), it is related to observed data. An example of this would be if a study records a history of dementia, and patients with dementia are more likely to forget to return a questionnaire. Data from the questionnaire would then be related to history of dementia i.e. observed data.

The last type of missing data is Missing Not At Random (MNAR), where the missing data is only related to unobserved factors. An example of this might be a sample that is missing because the patient had become unwell since the last record in a study and therefore missed having the sample taken.

A variety of techniques can be used for missing data, summarised in Table 3-5. A complete case analysis excludes subjects missing any data but this is inefficient and can lead to biased estimates if

data is not MCAR. The Last Observation Carried Forward replaces the missing data with the previous data, but this significantly distorts means, distorting inferences, and variance/covariance, distorting significance testing.

Table 3-5: Techniques for Handling Missing Data

Complete Case Analysis
Last Observation Carried Forward
Imputation Methods
• Simple Mean Imputation
• Regression Mean Imputation
• Multiple Imputation
Available Case Analysis
• Likelihood Models

Imputation methods involve substituting missing values with values derived from another method. Simple techniques include simple mean imputation where the mean of a variable is used, but this produces an underestimate of variance, and tends to dilute measures of association. An improvement on this is regression mean imputation where the variable with missing data is regressed on another, and the expected value of the missing data is calculated and imputed. This produces unbiased means, measures of association and regression coefficients, but the variance remains underestimated so significance testing can be biased.

Multiple imputation is a further development where the variable with missing data is regressed on other variables, using the results to inform a probability distribution from which a random value is drawn. This is repeated a number of times and the final analysis will be performed on each imputed dataset, with pooled estimates drawn. Estimates are less biased, but results are dependent on the

assumptions made and all imputation methods still rely on invented data. This technique was considered for use in chapter 4, but discarded as the final variables selected contained sufficiently small amounts of missing data to proceed with an available case approach.

For available case analysis, all data is retained in the analysis and if likelihood estimates of coefficients are used, the resulting parameters are unbiased in the case of MAR and MCAR.

3.4.5 Statistical versus Biological or Clinical Significance

As with all studies, it is important to distinguish between results that are statistically or biologically significant. This is particularly true when studies are large as the statistical power can be large, e.g. the correlations in chapter 5 (some of which are 'statistically significant' but quite weak correlations) and the multilevel analyses in chapter 4. The survival analyses in the thesis are less affected by this as the statistical power comes from the event number, and this is substantially less than total number of study participants.

Conversely, there are many benefits to large studies as well. It is possible to perform meaningful mortality analyses on the same population as used for other analyses, as in chapter 4. This is despite the fact that statistical power for survival analyses relies on event numbers (i.e. deaths) which are inevitably smaller than the total number of study participants. With large studies there will be greater precision in estimates of effect sizes. The increase in statistical power with large studies can compensate for any decrease in power arising from regression dilution bias due to measurement error, (170) such as may occur when samples have been shipped then stored for long periods of time. With large sample sizes, regression models can adjust for a far wider range of covariates, thereby improving the chances of identifying 'true' independent predictors of a given outcome.

4 Local versus systemic inflammation in peritoneal dialysis

4.1 Summary

4.1.1 Background

Systemic inflammation, as evidenced by elevated inflammatory cytokines, is a recognised feature of advanced renal failure and predicts worse survival. Dialysate IL-6 concentrations are associated with variability in peritoneal small solute transport rate (PSTR) which has also been linked to patient survival. The purpose of this analysis is to determine the link between systemic and intra-peritoneal inflammation and establish their relation to membrane function and patient survival.

4.1.2 Methods and Materials

The Global Fluid Study is a multi-national, multicentre, prospective, combined incident and prevalent (n=959 patients) cohort study with up to 8 years follow-up. Data collection included detailed demography, comorbidity, modality, prescription and membrane function. Dialysate and plasma cytokines were measured by electrochemiluminescence.

4.1.3 Results

426 survival endpoints occurred in 559 incident and 358 prevalent patients from 10 centres in Korea, Canada and the UK. On entry to the study there was dissociation between systemic and intra-peritoneal cytokine networks with evidence of local production within the peritoneum. After adjustment for multiple covariates, systemic inflammation was associated with age and comorbidity and was an independent predictor of patient survival in both incident and prevalent cohorts. In contrast, intra-peritoneal inflammation was the most important determinant of PSTR but did not affect survival. In prevalent patients the relationship between local inflammation and membrane function persisted but did not account for an increased mortality associated with faster PSTR.

4.1.4 Discussion

Systemic and local intra-peritoneal inflammation reflect distinct processes and consequences in patients treated with peritoneal dialysis, so their prevention may require different therapeutic approaches; the significance of intra-peritoneal inflammation requires further elucidation.

4.2 Introduction

Individual differences in peritoneal membrane function have been shown to influence clinical outcomes in peritoneal dialysis (PD) patients. In particular a high peritoneal solute transport rate (PSTR) has been linked to worse survival. (32,71) This association has been considered to be due to one of two main mechanisms – less efficient ultrafiltration and excess fluid reabsorption as a consequence of early loss of the glucose gradient during the dialysis dwell (68) or because high PSTR is a manifestation of the systemic inflammation commonly seen in advanced kidney failure.(167,171)The picture is further complicated by changes in PSTR due to acquired membrane injury with time on PD, (41) where in addition to reducing ultrafiltration by the above mechanisms it can be associated with a reduction in membrane efficiency (reduced osmotic conductance).(20)

More recently it was shown that PSTR is associated with the amount of IL-6 in drained dialysate, which is present in higher concentrations than can be explained by diffusion from plasma, implying its local production.(99,101) Furthermore, individuals with genetic polymorphisms associated with increased IL-6 production, both systemically and locally, have increased PSTR (37,38) and worse survival.(172) Against this association being the main link between PSTR and survival is the observation that it is confined to patients treated with continuous ambulatory PD (71) whereas in more recent cohorts where automated PD (APD) predominates the effect disappears (173,174) or even reverses. (78)

To date there are no studies linking dialysate cytokine profiles to survival and only small studies suggesting that dialysate IL-6 appearance reflects a wider activation of the local cytokine network.(101,175) The purpose of this first major analysis of the Global Fluid Study was to test the following hypotheses (a) that intra-peritoneal and systemic inflammation are distinct entities, (b) that it is local not systemic inflammation that associates with membrane function (PSTR), (c) that different clinical factors associate with local and systemic inflammation, and (d) that systemic but not local inflammation predicts patient survival.

4.3 Methods and Materials

4.3.1 Study design

The Global Fluid Study is an international, multi-centre, prospective, observational cohort study designed to answer a series of research questions seeking to relate peritoneal membrane function to local and systemic biomarkers as predictors of predefined clinical endpoints (e.g. patient survival, membrane injury). It was open to any centre worldwide as advertised at international meetings. 10 centres from the UK, Korea and Canada were finally included (see table 1 in supplementary material) in this analysis. An additional 6 centres (comprising 247 patients) were excluded based on a pre-analysis assessment indicating poor data quality (more than 10 variables were missing more than 10% of data) and it was judged unlikely that this could be improved upon due to logistic issues. Recruiting incident (within first 90 days of PD) and prevalent patients, enrolment commenced in June 2002, and finished in December 2008 (with some centres stopping before then), with follow-up censored at centre-specific dates during December 2010. Any patient on peritoneal dialysis was eligible for inclusion provided they could give informed consent. The sample size was the maximum logistically feasible, as determined by each centre. Dialysate sampling was from a 4 hour peritoneal equilibration test (PET), with some centres also collecting samples from an overnight dwell. Simultaneous clinical data were collected and stored in a purpose built Peritoneal Dialysis Access database (PDDB). Ethical approval was obtained from the Multi-Centre Research Ethics Committee for Wales covering the UK, whilst local country ethics were obtained for other contributing countries.

4.3.2 Prospective collection of routine clinical measurements

Routine demography was recorded and comorbidity documented using the validated Stoke Comorbidity Index that both categorises patients into low (score 0), intermediate (score 1-2), and high (score >2) risk groups, and enables analysis by individual comorbidities within the index. Patient level ethnicity was not available so this was recorded as non-Korean vs Korean based on centre.

Routine blood, urine and dialysate tests were performed locally and, if necessary, converted into standardised SI units.

PD related measurements included residual renal function (mean of urea and creatinine clearances), dialysis regime and dose, and peritoneal membrane function using the peritoneal equilibration test (solute transport rate: dialysate to plasma creatinine ratio (PSTR) and net UF capacity at 4 hours with 2.27% or 3.86% glucose, corrected for flush volume, if included in the measurement). The glucose exposure rate was calculated as total grams of glucose within the daily dialysate, and the average daily glucose concentration was the total daily dialysate glucose/total daily dialysate volume (grams/litre).

4.3.3 Sample analysis

Dialysate and plasma samples were stored locally at -80^{0}C, then transferred frozen to a central laboratory in the UK. Plasma and 4 hour dialysate samples were assayed for IL-1β, TNF-α, IFN-γ and IL-6 by electrochemiluminescence immune assay, using the commercially available Pro-Inflammatory I 4-plex (Meso-Scale Discovery, Gaithersburg, Maryland, USA). Triplicate measurements were made, the mean of which was used.

4.3.4 Statistical analysis

Demographic features were compared with independent sample t-tests, Mann-Whitney U tests or chi-squared tests, depending on whether the variable was normally distributed, skewed or categorical. Similarly for centre effects, one-way ANOVA or Kruskal-Wallis was used (table 1).

Pearson's R was used for cytokine correlations with Sidak's adjustment for multiple comparisons and a p value of 0.05 for statistical significance. The 3 pore model was used to predict 4 hour cytokine D/P ratios based on the predicted molecular radius**.** (13) For plasma values of 0 with detectable dialysate cytokine, a ratio greater than 1 was assumed; if both dialysate and plasma cytokine were undetectable, a ratio of 0 was assumed.

3 multilevel linear models for predictors of the continuous variables PSTR, and $\log_{10}$ transformations of dialysate and plasma IL-6 concentrations in 3 separate models were run to account for the observed centre effects by introducing a centre level residual as well as the usual person level residual. As an exploratory analysis, no adjustment of significance levels was made for multiple hypotheses tested. Random intercept models were fitted, (random slopes models were attempted but did not converge). The variable selection method was to include all cytokine measures and all the important clinical and available demographic variables. Dialysate IL-1β was dropped and only 1 measure of BP included due to multi-collinearity. Diabetes and comorbidity were included in separate models as existing literature suggests diabetic effects may be important independently of the comorbidity score, despite being highly correlated. (26) The duration of PD was included as either a linear or linear plus quadratic term in the incident group, as suggested by existing literature. (31) The Iterative Generalised Least Squares method was used for coefficient estimation and residuals were checked for normality. For clarity of interpretation, 23 patients with a previous episode of PD were excluded from the prevalent group multilevel modelling.

We included cytokine results in the PSTR model as either concentrations (as shown) or appearance rates, and selected the type that provided the best goodness of fit as measured by -2LL values. The selected variable type was then used in all other analyses.

Cox modelling, stratified by centre, was used for survival analysis, with robust standard errors. Hazard Ratios for cytokines quoted are for a $\log_{10}$ change in concentration. Proportional hazards were checked with log-log plots, scaled Schoenfeld residual plots and significance testing. Dialysate IL-1β was excluded due to high collinearity.

MLWin v2.26 (Centre for Multilevel Modelling, Bristol University) was used for the multilevel modelling. All other analyses were run using Stata IC 12.1 (StataCorpLP, Texas). Missing data, which ranged between 0 to 4.8% for different variables, were considered missing at random and complete

case analysis was used. Loss to follow up was trivial (16 incident and 8 prevalent patients, with 22 from one centre and all patients from that centre being dropped in a sensitivity analysis).

4.4 Results

4.4.1 Description of incident and prevalent cohorts

The clinical characteristics of the 959 patients included in the analyses are shown in Table 4-2. Comparing the incident and prevalent groups the latter used more icodextrin and APD, greater total dialysate volumes and had lower urine volume. Although the use of APD was relatively low, this was much more likely to be prescribed in patients with faster PSTR (0.78 v 0.7, P=0.0005).

For most patient characteristics and prescription practices there were highly significant centre effects (Table 4-1) and for this reason all linear regression models used multi-level methods.

Table 4-1: Intra-cluster correlations for PSTR, dialysate and plasma IL-6

Variable	Incident	Prevalent
PSTR	0.17 (0.09, 0 – 0.34)	0.17 (0.09, 0 – 0.35)
Dialysate IL-6	0.18 (0.09, 0 – 0.36)	0.09 (0.08, 0 – 0.32)
Plasma IL-6	0.08 (0.05, 0 – 0.17)	0.005 (0.016, 0 – 0.037)

Data presented = ICC (SE, 95% CI)

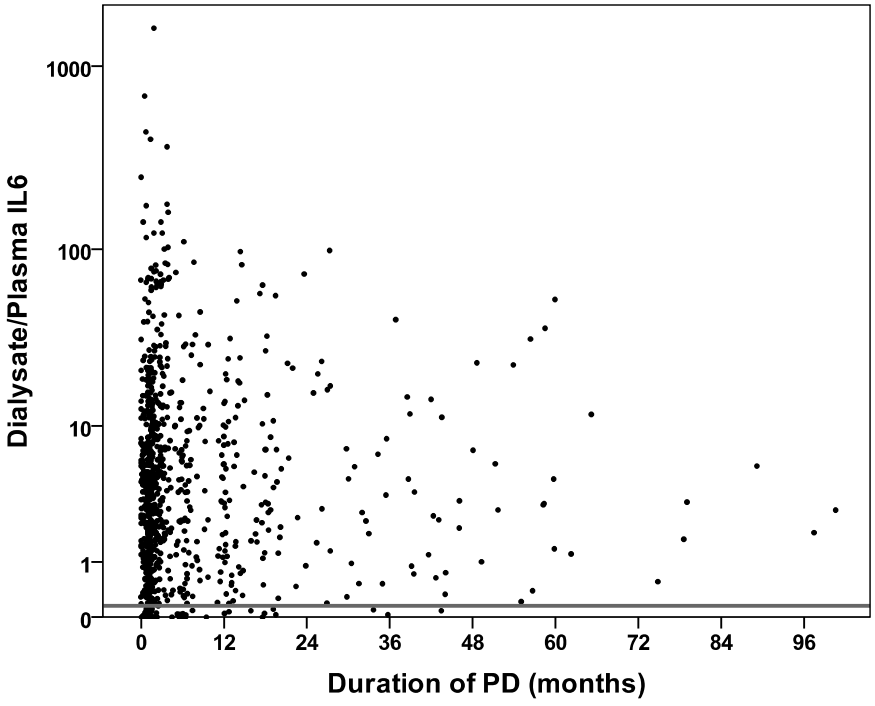
Table 4-2: Study Population Characteristics

	Incident N = 575	Centre Effect (p value)	Prevalent N=384	Centre Effect (p value)	Difference between incident and prevalent (p value)
Age (years)	55.6 (15.3)	0.001	54.2 (15.2)	0.037	NS
Female Gender	38.4%	NS	46.4%	NS	0.05
Korean	37.2%	By definition	36.2%	By definition	NS
BMI (kg/height^2)	25.2 (4.7)	<0.001	25.3 (4.7)	<0.001	NS
Total dialysate volume (litres)	7.96 (1.29)	<0.001	8.38 (1.87)	<0.001	<0.001
Blood pressure (mmHg)	136/80 (21/12)	<0.001	135/81 (20/12)	NS	NS
Duration of PD (days; median)	40 (28, 55)	<0.001	360 (169, 609)	<0.001	<0.001
4 hour PSTR	0.71 (0.12)	<0.001	0.71 (0.12)	<0.001	NS
UF capacity	431 (365)	<0.001	439 (340)	<0.001	NS
Albumin	35.0 (5.2)	<0.001	35.4 (4.8)	0.06	NS
Haemoglobin	11.0 (2.2)	<0.001	11.2 (1.8)	<0.001	NS
Urine volume (litres; median)	0.90 (0.46, 1.44)	<0.001	0.60 (0.19, 1.21)	0.001	<0.001
Biocompatible solution usage	19.3%	<0.001	16.1%	<0.001	NS
Icodextrin solution usage	19.1%	<0.001	28.0%	<0.001	0.002
Comorbidity (Low/Intermediate/High)	35.6/56.8/7.6%	<0.001	43.9/48.5/7.6%	<0.001	NS
Use of APD	6.0%	<0.001	15.1%	<0.001	<0.001

4.4.2 Demonstration that local peritoneal and systemic inflammation is partly uncoupled

To establish that dialysate IL-6 is representative of a localised inflammatory process is it necessary to demonstrate both local production and an association with other pro-inflammatory cytokines that is independent of plasma. Taking molecular size into account, 87% of subjects had dialysate IL-6 concentrations higher than predicted by diffusion across the peritoneal membrane (Figure 4-1). Values for IL-1, TNF-α and IFN-γ were 33.3%, 6.9% and 45.7% respectively. Within the peritoneal and circulatory compartments there were moderate to strong correlations between the measured cytokines reflecting localised activation of pro-inflammatory networks (Table 4-3). In contrast, correlations between dialysate and plasma were either absent or weaker than those seen within plasma or dialysate.

Figure 4-1: Scatterplot of Dialysate/Plasma Concentration Ratio of IL-6 Against Duration of PD



The line represents the ratio predicted by the 3 pore model (0.145) so all points above this line are predicted to represent local production.

Table 4-3: Correlation Coefficients Between Cytokine Concentrations

Incident		Dialysate				Plasma		
		IL-1β	**IFN-γ**	**IL-6**	**TNF-α**	**IL-1β**	**IFN-γ**	**TNF-α**
Dialysate (n=563)	**IFN-γ**	**0.65**						
	IL-6	**0.29**	**0.29**					
	TNF-α	**0.82**	**0.74**	**0.42**				
Plasma (n=557)	**IL-1β**	-0.004	0.005	-0.01	-0.07			
	IFN-γ	0.0002	-0.01	0.11	-0.007	0.10		
	TNF-α	-0.05	-0.01	0.15*	-0.03	0.08	**0.51**	
	IL-6	0.05	0.09	**0.28**	0.04	0.13*	**0.25**	**0.35**
Prevalent								
Dialysate (n=378)	**IFN-γ**	**0.61**						
	IL-6	**0.21**	**0.32**					
	TNF-α	**0.76**	**0.75**	**0.43**				
Plasma (n=379)	**IL-1β**	0.12	0.10	-0.02	0.09			
	IFN-γ	0.04	0.05	-0.001	0.05	0.12		
	TNF-α	-0.07	-0.06	0.11	0.01	0.14	**0.43**	
	IL-6	-0.02	0.11	**0.27**	0.07	0.15	**0.24**	**0.29**

Sidak adjusted p values ≤0.001 in bold, * p 0.01-0.05, otherwise p>0.05. There were 548 and 374 common observations between dialysate and plasma samples for incident and prevalent patients respectively.

4.4.3 Local not systemic inflammation is the main determinant of PSTR

Results of the multivariable, multilevel, linear regression models showing the associations with PSTR are displayed in table 3. Dialysate IL-6 concentration was the most significant association in both patient cohorts, a pattern observed in all of the participating centres. This was independent of patient factors (gender, race, BMI, BP, urine volume, diabetic status) and dialysis prescription, all of which had significant associations. For incident patients, the timing of the initial membrane function assessment had an effect that was not linear: tests done early, i.e. from baseline the PSTR rose for 2 months, increasing by 0.08, then fell to a total gain of 0.06 by 3 months. In prevalent patients, higher PSTR was associated in a linear fashion with longer time on treatment. Cytokine concentrations produced a better model than appearance rates (Δ-2 LL = 36).

Table 4-4: Predictors of PSTR

	Incident		**Prevalent**	
	Coefficient (95% CI)	**p value**	**Coefficient (95% CI)**	**p value**
Age (per decade)	0.001 (-0.005, 0.008)	0.7	-0.004 (-0.012, 0.004)	0.4
BMI	**-0.002 *** (-0.005, -0.0001)	0.04	-0.0009 (-0.004, 0.002)	0.5
APD usage	-0.02 (-0.06, 0.02)	0.3	-0.008 (-0.04, 0.03)	0.7
Systolic BP (per 10mmHg)	**0.005 *** (0.0002, 0.009)	0.04	0.001 (-0.004, 0.007)	0.6
Male Gender	**0.02 *** (0.003, 0.04)	0.02	**0.02 *** (0.002, 0.05)	0.04
Duration of PD	**0.08 x month **** (0.03, 0.13), **-0.02 x month^2 *** (-0.04, -0.003)	<0.001	**0.01 x year **** (0.004, 0.02)	0.003
Biocompatible Solution Usage	-0.005 (-0.02, 0.02)	0.7	**-0.04 *** (-0.07, (-0.004)	0.03
Icodextrin Usage	**0.06 **** (0.03, 0.09)	<0.001	**0.04 *** (0.01, 0.07)	0.01
Average glucose concentration (per gramme/litre)	**0.005 **** (0.002, 0.007)	<0.001	**0.005 **** (0.001, 0.008)	0.004
Dialysate IL-6	**0.08 **** (0.06, 0.11)	<0.001	**0.09 **** (0.07, 0.12)	<0.001
Dialysate TNF-α	0.04 (-0.03, 0.10)	0.3	-0.03 (-0.1, 0.06)	0.6
Dialysate IFN-γ	-0.009 (-0.04, 0.02)	0.6	0.008 (-0.03, 0.04)	0.6
Plasma IL-6	-0.02 (-0.06, 0.01)	0.2	0.006 (-0.04, 0.05)	0.8
Plasma TNF-α	0.02 (-0.04, 0.09)	0.5	-0.05 (-0.12, 0.02)	0.2
Plasma IFN-γ	-0.009 (-0.04, 0.02)	0.6	-0.02 (-0.06, 0.03)	0.4
Plasma IL-1β	0.02 (-0.06, 0.11)	0.6	0.001 (-0.09, 0.09)	0.98
Diabetic	**0.02 *** (0.001, 0.05)	0.04	0.004 (-0.03, 0.03)	0.8
Comorbidity	0.0005 (-0.01, 0.01)	0.9	0.003 (-0.01, 0.02)	0.7
Urine volume (per litre)	**0.03 **** (0.01, 0.04)	<0.001	**0.02 *** (0.005, 0.04)	0.01
Korean	**0.08 *** (0.01, 0.15)	0.02	0.05 (-0.005, 0.11)	0.07

*p<0.05 **p<0.005

4.4.4 Determinants of local versus systemic inflammation

Before proceeding to survival analyses it was necessary to determine, using the multilevel multivariable models, the clinical associations with local (Table 4-5) and systemic (Table 4-6) inflammation as defined by the dialysate and plasma IL-6 concentrations respectively.

Local membrane inflammation was associated with older age, lower systolic blood pressure, use of icodextrin, local TNF-α and systemic IL-6 concentrations in incident and prevalent patients. Factors associated with systemic inflammation were similar where a reciprocal effect might be expected (e.g. the plasma and dialysate cytokines) but also included a relationship with comorbidity; in prevalent patients this was with the overall comorbid burden whereas in prevalent patients it was especially evident with diabetics (although the IL-6 levels were still different between the grades of comorbidity, one-way between subjects ANOVA, p=0.006).

Sensitivity analyses excluding one centre with marginally less good data quality increased the significance of the association between plasma IL-6 and age (p=0.02) in prevalent patients. A supplementary analysis including PSTR as one of the covariates is included in Appendix A (Chapter 11.1)

Table 4-5: Predictors of Dialysate IL-6

	Incident		Prevalent	
	Coefficient	**p value**	**Coefficient**	**p value**
Age (per decade)	**0.04 **** (0.01, 0.06)	0.002	**0.05 **** (0.01, 0.08)	0.01
BMI	0.0009 (-0.007, 0.009)	0.8	0.008 (-0.003, 0.02)	0.2
APD Usage	-0.06 (-0.21, 0.10)	0.5	0.2 (-0.004. 0.3)	0.06
Systolic BP (per 10mmHg)	**-0.02 *** (-0.03, -0.002)	0.03	**-0.03 **** (-0.05, -0.002)	0.03
Male Gender	0.04 (-0.04, 0.11)	0.3	0.1 (-0.0003, 0.2)	0.051
Duration of PD (per year)	0.1 (-0.6, 0.8)	0.7	0.02 (-0.02, 0.05)	0.4
Biocompatible solution usage	0.0007 (-0.09, 0.09)	0.99	0.1 (-0.04, 0.3)	0.1
Icodextrin Use	**0.3 **** (0.2, 0.4)	<0.001	**0.2 **** (0.07, 0.3)	0.003
Average Glucose Concentration (per gramme/litre)	**0.01 **** (0.004, 0.02)	0.006	-0.004 (-0.02, 0.01)	0.5
Dialysate TNF-α	**0.8 **** (0.6, 1.0)	<0.001	**0.7 **** (0.3, 1.0)	0.001
Dialysate IFN-γ	0.006 (-0.1, 0.1)	0.9	0.02 (-0.1, 0.2)	0.8
Plasma IL-6	**0.3 **** (0.2, 0.4)	<0.001	**0.3 **** (0.1, 0.5)	0.001
Plasma TNF-α	-0.2 (-0.4, 0.06)	0.1	0.2 (-0.1, 0.5)	0.2
Plasma IFN-γ	0.06 (-0.06, 0.19)	0.3	-0.1 (-0.3, 0.03)	0.1
Plasma IL-1β	-0.1 (-0.3, 0.3)	0.9	-0.05 (-0.5, 0.4)	0.8
Diabetic	0.01 (-0.08, 0.10)	0.8	0.05 (-0.08, 0.2)	0.4
Comorbidity	0.02 (-0.03, 0.06)	0.5	-0.004 (-0.06, 0.05)	0.9
Urine volume (per litre)	0.03 (-0.02, 0.08)	0.2	**-0.1 *** (-0.2, -0.02)	0.01
Korean	-0.02 (-0.2, 0.2)	0.8	-0.2 (-0.5, 0.1)	0.3

Table 4-6: Predictors of Plasma IL-6

	Incident		Prevalent	
	Coefficient	**p value**	**Coefficient**	**p value**
Age (per decade)	**0.02 **** (0.007, 0.04)	**0.004**	0.01 (-0.009, 0.03)	0.3
BMI	0.0001 (-0.005, 0.005)	0.96	0.004 (-0.002, 0.01)	0.2
APD Usage	0.04 (-0.06, 0.14)	0.4	0.003 (-0.08, 0.09)	0.9
Systolic BP (per 10mmHg)	0.003 (-0.007, 0.014)	0.5	-0.009 (-0.02, 0.004)	0.2
Male Gender	0.05 (-0.001, 0.09)	0.06	0.02 (-0.03, 0.07)	0.5
Duration of PD (per year)	-0.2 (-0.6, 0.2)	0.4	**0.02 *** (0.0008, 0.03)	**0.04**
Biocompatible solution usage	0.003 (-0.05, 0.06)	0.9	-0.02 (-0.10, 0.06)	0.6
Icodextrin usage	0.04 (-0.02, 0.11)	0.3	-0.02 (-0.09, 0.05)	0.6
Average Glucose Concentration (per gramme/litre)	0.003 (-0.004, 0.009)	0.4	-0.002 (-0.01, 0.005)	0.5
Dialysate IL-6	**0.13 **** (0.07, 0.18)	**<0.001**	**0.09 **** (0.03, 0.15)	**0.002**
Dialysate IFN-γ	0.07 (-0.003, 0.15)	0.06	0.04 (-0.04, 0.1)	0.3
Dialysate TNF-α	**-0.2 *** (-0.3, -0.005)	**0.04**	-0.1 (-0.4, 0.06)	0.2
Plasma TNF-α	**0.4 **** (0.2, 0.5)	**<0.001**	**0.4 **** (0.2, 0.5)	**<0.001**
Plasma IFN-γ	0.05 (-0.03, 0.13)	0.2	**0.2 **** (0.07, 0.3)	**0.001**
Plasma IL-1β	**0.2 *** (0.001, 0.4)	**0.049**	**0.3 *** (0.07, 0.5)	**0.01**
Diabetic	-0.05 (-0.1, 0.007)	0.09	**0.07 *** (0.002, 0.15)	**0.045**
Comorbidity	**0.05 **** (0.02, 0.08)	**0.001**	0.02 (-0.01, 0.05)	0.2
Urine Volume (per litre)	-0.02 (-0.05, 0.01)	0.3	-0.009 (-0.05, 0.03)	0.7
Korean	0.03 (-0.05, 0.11)	0.4	-0.04 (-0.1, 0.1)	0.4

4.4.5 Systemic not local inflammation predicts patient survival

A total of 427 deaths occurred in the two cohorts (241 and 186 in the incident and prevalent groups respectively during median follow up times of 5.25 and 5.06 years) during the 8 year follow-up period. Using Cox modelling, survival of incident patients was independently predicted by age, cumulative comorbidity, plasma albumin, and systemic inflammation (IL-6 and TNF-α), whereas dialysate cytokines levels and PSTR had no effect (Table 4-7). Survival analysis in the prevalent group also found age, comorbidity, systemic IL-6 but not peritoneal inflammation, to predict death, with some additional differences. Plasma albumin did not predict survival whereas residual renal function was protective and a faster PSTR was associated with increased mortality. Sensitivity analyses excluding one centre with higher levels of missing data increased the significance of PSTR in prevalent patients (p=0.02) and excluding patients with previous episodes of PD treatment from the prevalent group made no difference.

Table 4-7: Predictors of Survival

	Incident		Prevalent	
	Hazard Ratio (95% CI)	**p value**	**Hazard Ratio (95% CI)**	**p value**
Dialysate TNF-α	0.96 (0.33, 2.82)	0.95	0.91 (0.23, 3.63)	0.9
Dialysate IL-6	0.93 (0.66, 1.31)	0.7	0.96 (0.64, 1.43)	0.8
Dialysate IFN-γ	1.19 (0.70, 2.02)	0.5	1.20 (0.65, 2.20)	0.6
Plasma IL-1β	0.58 (0.15, 2.19)	0.4	0.54 (0.16, 1.77)	0.3
Plasma TNF-α	**3.41 *** (1.26-9.24)	**0.02**	2.03 (0.52, 7.93)	0.3
Plasma IL-6	**2.16 **** (1.23, 3.80)	**0.007**	**2.69 **** (1.29, 5.59)	**0.008**
Plasma IFN-γ	0.86 (0.49, 1.50)	0.6	1.20 (0.65, 2.21)	0.6
Age (per year)	**1.06 **** (1.05, 1.08)	**<0.001**	**1.06 **** (1.04, 1.07)	**<0.001**
Male Gender	0.93 (0.69, 1.27)	0.7	1.28 (0.92, 1.79)	0.1
Comorbidity (per disease)	**1.68 **** (1.44, 1.97)	**<0.001**	**1.37 **** (1.18, 1.58)	**<0.001**
Urine volume (per litre)	0.95 (0.76, 1.19)	0.7	**0.65 **** (0.48, 0.87)	**0.004**
Duration of PD (per month incident) (per year prevalent)	1.05 (0.80, 1.19)	0.7	**1.14 **** (1.04, 1.24)	**0.005**
Albumin (per 1 g/dl)	**0.94 **** (0.91, 0.97)	**<0.001**	0.99 (0.95, 1.03)	0.6
PSTR (per 0.1 increase in D/P Cr)	1.09 (0.98, 1.23)	0.1	**1.18 *** (1.003, 1.41)	**0.046**
BMI	1.01 (0.97, 1.05)	0.6	1.01 (0.98, 1.04)	0.6

4.5 Discussion

This analysis of the Global Fluid Study clearly shows that systemic and local peritoneal inflammatory cytokine networks are to some extent uncoupled and that they have different consequences for patient survival. Local, subclinical peritoneal inflammation is demonstrated to be the strongest known factor associated with between patient variability in PSTR, independent of centre effects, and the lack of an association with survival refutes the prior hypothesis that fast PSTR increases mortality through its association with systemic inflammation. If anything, evidence points to intra-peritoneal inflammation being a contributor to systemic inflammation without influencing its association with mortality.

Although the association between local inflammation and PSTR has been found in prior studies (99,101,175) none of these has had either the power or the degree of detailed clinical data to show its relative importance compared to previously demonstrated, much weaker, clinical associations. As with CANUSA,(176) ANZDATA(26) and the Stoke PD Study, (17,41) diabetics and males were found to have higher PSTR, whereas the association with increasing age, overall comorbidity and inverse relationship to BMI were not seen. This is also the first study to identify important centre effects and include adjustment for these in the analytic approach. These centre effects will reflect differences such as case mix and race, practice patterns related to dialysis and EPO prescription which could largely be adjusted for, but also differences in PSTR that are likely to reflect local variations in exactly how the peritoneal equilibration test (PET) is performed or biochemically analysed, as well as unknown factors. Timing of the initial PET showed a complex relationship from which it is possible to infer that there is an early increase in PSTR within the first four weeks of treatment with a subsequent fall before a longer term increase in keeping with previous reports.(17,30,31) Given the ANZDATA's finding that race influenced PSTR (26) it is interesting that this was found to be higher in Korean patients, independent of IL-6 levels, suggesting that other genetic factors might be important.

It is difficult to disentangle the observed association between use of either icodextrin or higher glucose concentration solutions with higher dialysate IL-6 concentrations given that they also associate with PSTR and thus there may be some confounding by indication. However, icodextrin in combination with other solutions has been associated with increased solute transport, (55,177) as have other biocompatible solutions at the commencement of treatment with PD.(50) One possible explanation is that more biocompatible dialysate improves local cell viability and thus facilitates the local production of cytokines or vaso-active mediators.(53,178) In light of the recently published balANZ study in which use of a biocompatible solution was associated with disappearance of the increase in solute transport with time on PD,(50) it is interesting to note in this study that prevalent patients using these solutions had lower PSTR.

The associations between plasma IL-6, other systemic inflammatory cytokines and comorbidity were to be expected and are in keeping with the previously described relationship between IL-6 polymorphisms, comorbidity and survival in haemodialysis and PD patients.(111,172) More surprising is the association between plasma and dialysate IL-6. This could reflect the fact that genetically high IL-6 producers more readily synthesise more of this cytokine in any of the body compartments.(37) Alternatively, the high concentrations in dialysate, which in some of these patients was >1000 times that of plasma despite the diluting effects of two litres of instilled solution, reflects peritoneal membrane concentrations that could spill over into plasma.

The relationship between systemic inflammation and survival, independent of age and comorbidity was as anticipated, although previous studies have not reported independent effects of TNF-α and IL-6 as observed here in incident patients.(179,180) There were other potentially important differences between the incident and prevalent cohorts, partly because prevalent patients are by definition a self-selected cohort. As would be expected, longer duration of PD was a risk factor for worse survival. Relative preservation of residual urine volume, in keeping with prior studies, is more important than for incident patients whereas it's likely that the patients with the lowest plasma

albumin concentrations will have already died explaining the lack of association with survival. It is interesting to note that in these prevalent patients increased solute transport was associated with reduced survival; this may be because the relative importance of membrane function would be expected to increase as residual function becomes more critical. Although the use of APD at the start of PD in the GFS cohort is relatively low, it was used preferentially in patients with high PSTR and with double the frequency in prevalent compared to incident patients. In contrast to most published cohorts, icodextrin use was high.

This study has a number of limitations. Despite the depth and completeness of the clinical data collected and attempts to account for important observed centre effects, it must be acknowledged that there are likely to be practice patterns and local factors that remain poorly understood and/or unmeasured along with other residual confounders. Whilst the study used 10 centres from 3 countries a degree of selection bias might be present as the selected centres had better data quality. As with any observational study, direction of causality must always be questioned. The genetic associations between high producing IL-6 polymorphisms and membrane function, effectively Mendelian randomisation experiments, strongly suggest that activation of local cytokine networks are the cause rather than the consequence of increased PSTR, which is also biologically plausible. However there were a number of statistical associations demonstrated that do not have clear biological explanations. For example, a lower systolic blood pressure was associated with higher dialysate but not plasma IL-6 concentration. These require reproducing in separate cohorts and further investigation. Despite clear evidence of local production in some patients, average dialysate TNF-α levels were less than predicted by the 3 pore model, but the results were biologically plausible and compatible with previous studies.(103) Cost and feasibility dictated that we limit our inflammatory cytokine profiles to just four; other studies using larger panels of biomarkers confirm that these are representative of activation of the inflammatory pathway in general. (101,175,181) Controversy exists as to whether dialysate biomarkers should be expressed as absolute concentrations or appearance rates. In our multivariable analysis, dialysate IL-6 concentrations

produced better models than appearance rates suggesting biological effects are determined by concentration, mediated by changes over log orders. Correcting for appearance rates produced worse models, probably because the dialysate samples were all standardised to 4 hour dwells and a recent study has shown a linear increase in IL-6 concentrations with time. (182)

In conclusion, dialysate IL-6 concentration, representing local subclinical intra-peritoneal inflammation is the most significant known predictor of PSTR, but does not determine patient survival. Intra-peritoneal and systemic inflammation are independent, except for an association in the case of IL-6. Independent of inflammation, higher PSTR may still be associated with worse survival in prevalent patients. The clinical implications of these findings are that attention to membrane function in dialysis prescription rather than switching off membrane inflammation per se is important for patient survival. The relevance of membrane inflammation is yet to be determined.

5 Longitudinal changes in inflammation and peritoneal solute transport

5.1 Summary

5.1.1 Background

The current literature on the longitudinal changes in PSTR, and peritoneal and systemic inflammation is confused, with studies sampling at different time points producing apparently contradictory results. The GLOBAL Fluid Study had samples from many time points, allowing an analysis without pre-supposed hypotheses about the nature of these changes.

5.1.2 Methods and Materials

We used the GFS, a multi-national, multicentre, prospective, combined incident and prevalent (n=959 patients) cohort study with up to 8 years follow-up. Data collection included demography and membrane function, with dialysate and plasma cytokines measured by electrochemiluminescence. PSTR and inflammatory cytokines were plotted against duration of PD with fractional polynomials and based on this, time was divided into 0-2, 2-18 and >18 months. Correlations were used to test the significance of these apparent changes over time.

5.1.3 Results

PSTR, systemic inflammatory cytokines and dialysate IL-6 increased between 0 and 2 months, dialysate inflammatory cytokines all fell between 2 and 18 months, and >18 months PSTR increased and plasma TNF-α fell.

5.1.4 Conclusions

The start of PD appears to be associated with an increase in systemic inflammation, possibly mediated by absorption of dialysate IL-6 and in the medium term, there appears to be a fall in peritoneal inflammation. These results are hypothesis generating.

5.2 Introduction

As discussed in the Introduction (Chapter 2.2.3.1) there are changes in PSTR with time on PD, and as we have confirmed with the GFS, dialysate IL-6, which is indicative of peritoneal inflammation, is the strongest predictor of PSTR but whether there are temporal changes in dialysate IL-6 to explain the changes in PSTR is not known.

PSTR increases from the first to the fourth week of PD**,** (30) but there may, (31) or not may not, (41) be a fall in PSTR from the first to the fifth or sixth months**.** Dialysate IL-6 has been examined at baseline and at 1 year by Pecoits-Filho et al (99) and at 1, 6 and 12 months by Cho et al, (100) but both studies found increases over these time frames, an apparently contradictory result given that dialysate IL-6 is the strongest known predictor of PSTR (Chapter 4). The Pecoits-Filho et al and Cho et al studies also analysed systemic IL-6 levels at these time points, finding a rise with time and stable levels respectively.

As the GFS did not specify when PET's with dialysate and plasma samples were to be taken, it represented an opportunity to study changes in both PSTR and inflammatory markers over time without examining them at arbitrary time points as has been done previously. This allows us to look for changes between these time points to try and reconcile the different findings.

5.3 Materials and Methods

All patients from the Global Fluid Study were included and the study design, routine clinical measurements and sample analysis are the same as in sections 3.3.1, 3.3.2 and 3.3.3 respectively. Patients were not divided into incident and prevalent cohorts but analysed together.

In an exploratory analysis, logarithmically transformed dialysate and plasma cytokines and D/P Cr were separately included in a fractional polynomial regression against the duration of PD using 3 polynomial terms. This allows modelling of the relationship without assuming a particular form (e.g. linear). Because of the scarcity of data at longer time points, this was restricted to the first 5 years of PD.

The patients were then divided into early (0-2 months), medium (2-18 months) and late (>18 months) groups, these divisions having been selected as representative of the nadirs and peaks in the apparent trends over time visible in the graphs, with the exact timing of the changes calculated from the polynomial regression equation. Pearson correlation coefficients were calculated for cytokine measurements and D/P Cr with the duration of PD based on these divisions. As this was an exploratory analysis, no correction was made for multiple tests.

To investigate an unexpected finding for plasma TNF-α, a multilevel multivariable model of log-transformed plasma TNF-α with centre for the level 2 and person for the level 1 residual was run, utilising MLwiN v2.26 (Centre for Multilevel Modelling, Bristol University) via runmlwin.

To further investigate a discrepancy between regression results and correlation coefficient for plasma IL-1β, both a fractional polynomial regression with 4 polynomial terms and a lowess regression were run.

All analyses were performed with Stata IC v12.1 (College Station, Texas).

5.4 Results

960 patients were included with demographic and clinical details shown in Table 5-1.

Table 5-1: Patient Details

	n=960
Age	55.0 (15.3)
BMI	25.3 (4.7)
BP	135.7/80.3 (20.8/12.2)
Albumin	35.1 (5.0)
Urine Volume (mls)	800 (309-1320)
Duration of PD (days)	63 (36-212)
Biocompatible Usage	18.10%
Icodextrin Usage	22.70%
APD Usage	9.70%
Comorbidity (Low/Intermediate/High)	38.9/53.5/7.6%
Korean	36.80%
Female	41.70%

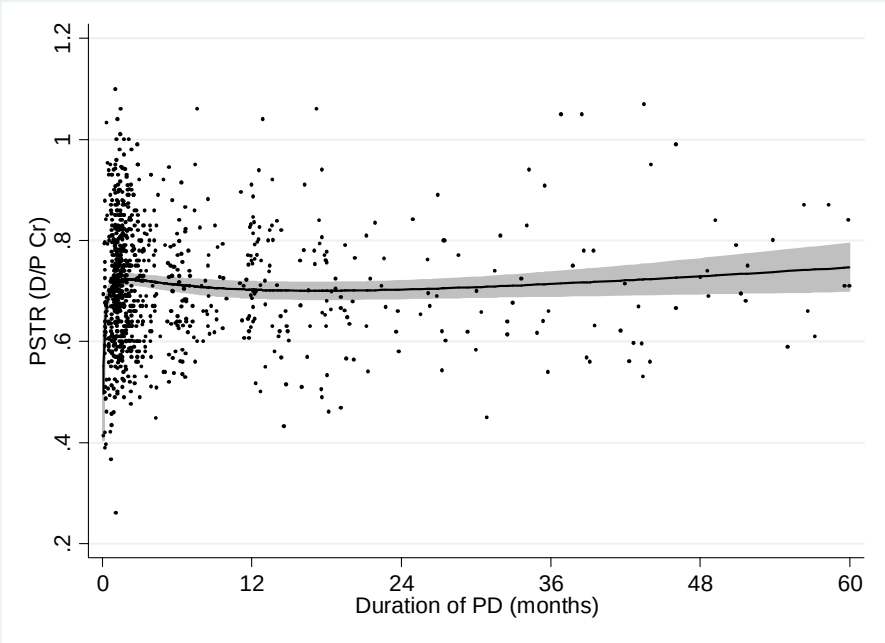
Results of the fractional poloynomial regression with 95% confidence intervals for PSTR, dialysate cytokines and plasma cytokines against duration of PD are shown in Figure 5-1, Figure 5-2, Figure 5-3, Figure 5-4, Figure 5-5, Figure 5-6, Figure 5-7, Figure 5-8 and Figure 5-9.

For both plasma TNF-α and IFN-γ, there was an initial apparent fall, but this was based solely on the first 2 slightly higher values. Otherwise the trends with time are shown in Table 5-2. To test the significance of these apparent trends, we split the duration of PD into early, middle and late time periods based on the data in Table 5-2, and calculated correlation coefficients as shown in Table 5-3. The correlations are not strong, with the largest value being 0.36 for the association of solute transport with time over 18 months.

The correlations based on these scatterplots are shown in Table 5-3, demonstrating an increase in systemic inflammation over the first two months of PD, along with dialysate IL-6 and PSTR, followed by a fall in dialysate cytokines over the next 16 months. In the longer term PSTR increases whilst

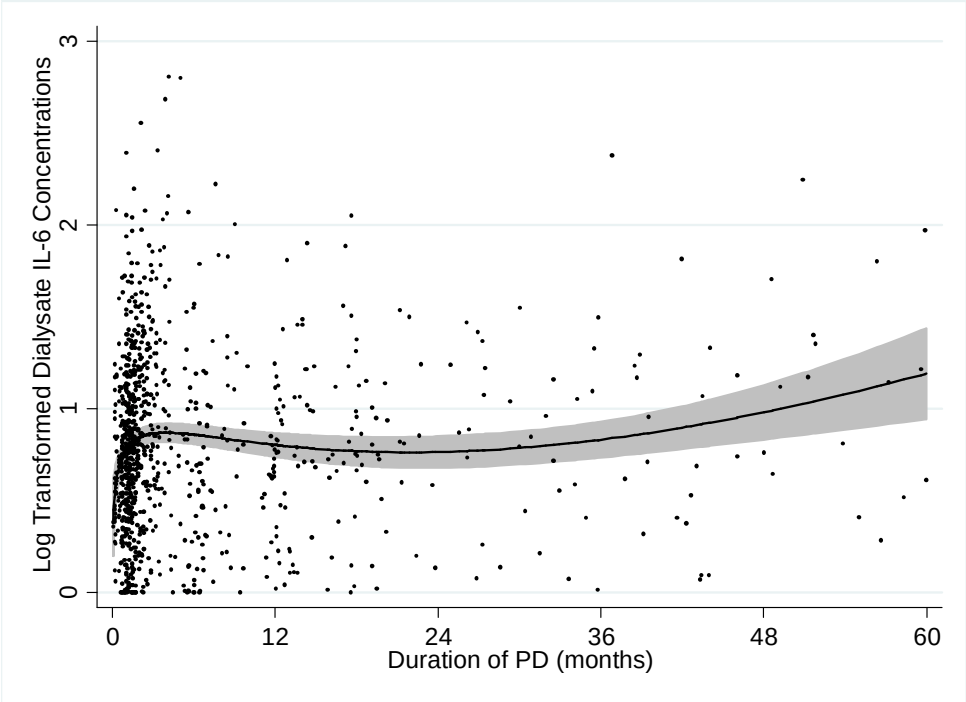
plasma TNF-α levels fall. Because plasma IL-6 did not fit the apparent pattern in systemic cytokines over the first 2 months despite the significant appearance of the regression, one further correlation of plasma IL-6 with duration of PD was performed limited to the first 45 days of PD (r=0.17, p=0.002).

Figure 5-1: Peritoneal Solute Transport Rate with Duration of PD



Black line represents fitted line, with grey area the 95% confidence interval for this, for Figure 5-1, Figure 5-2, Figure 5-3, Figure 5-4, Figure 5-5, Figure 5-6, Figure 5-7, Figure 5-8 and Figure 5-9.

Figure 5-2: Dialysate IL-6 By Duration of PD



Black line represents fitted line, with grey area the 95% confidence interval for this.

Figure 5-3: Dialysate IL-1β By Duration of PD

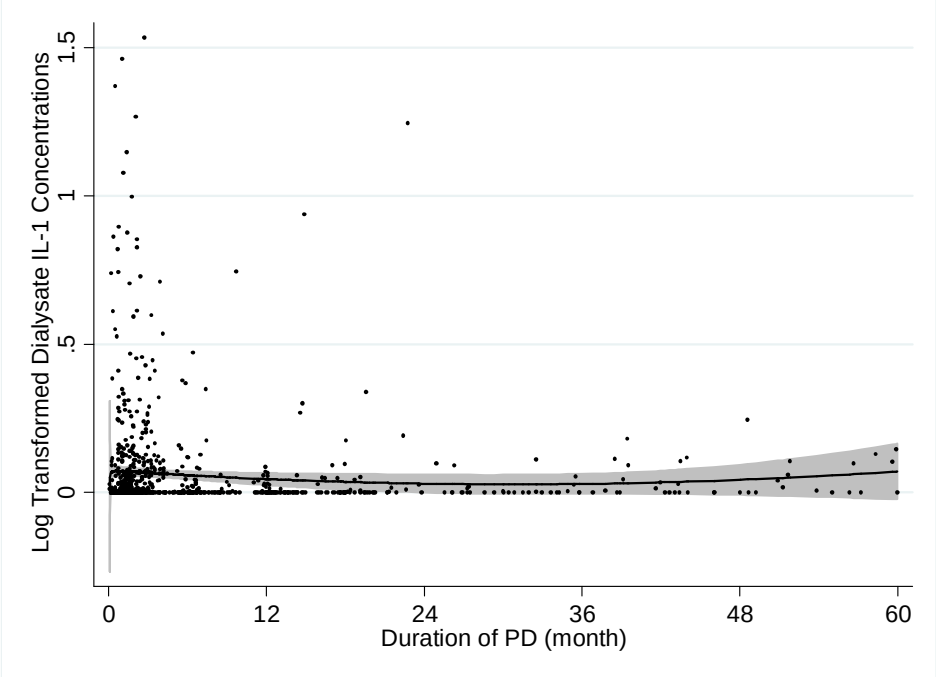


Figure 5-4: Dialysate IFN-γ By Duration of PD

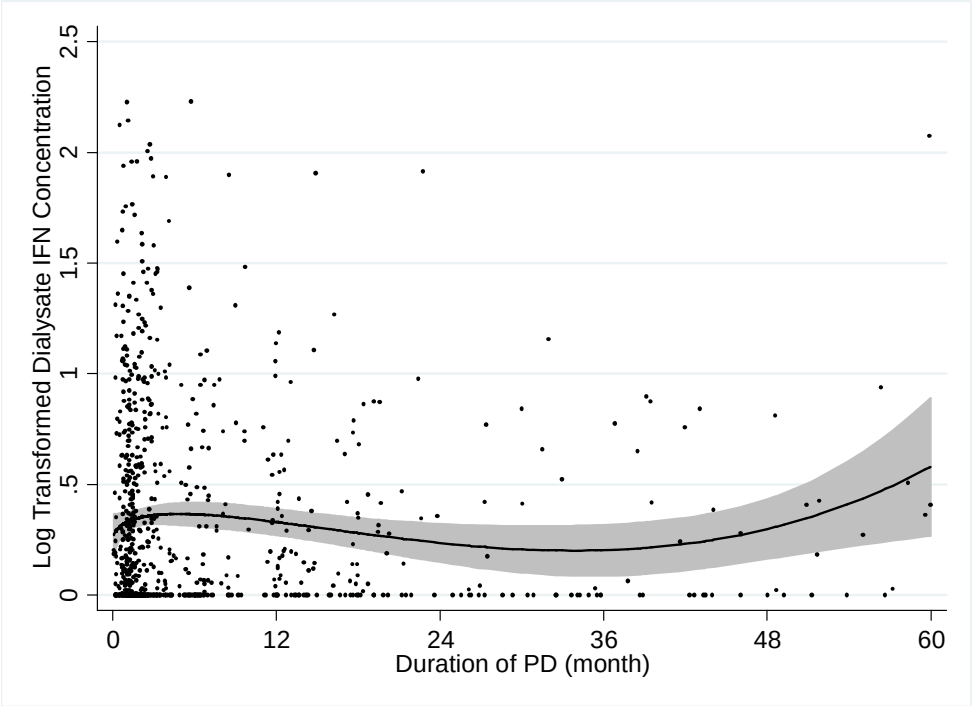


Figure 5-5: Dialysate TNF-α By Duration of PD

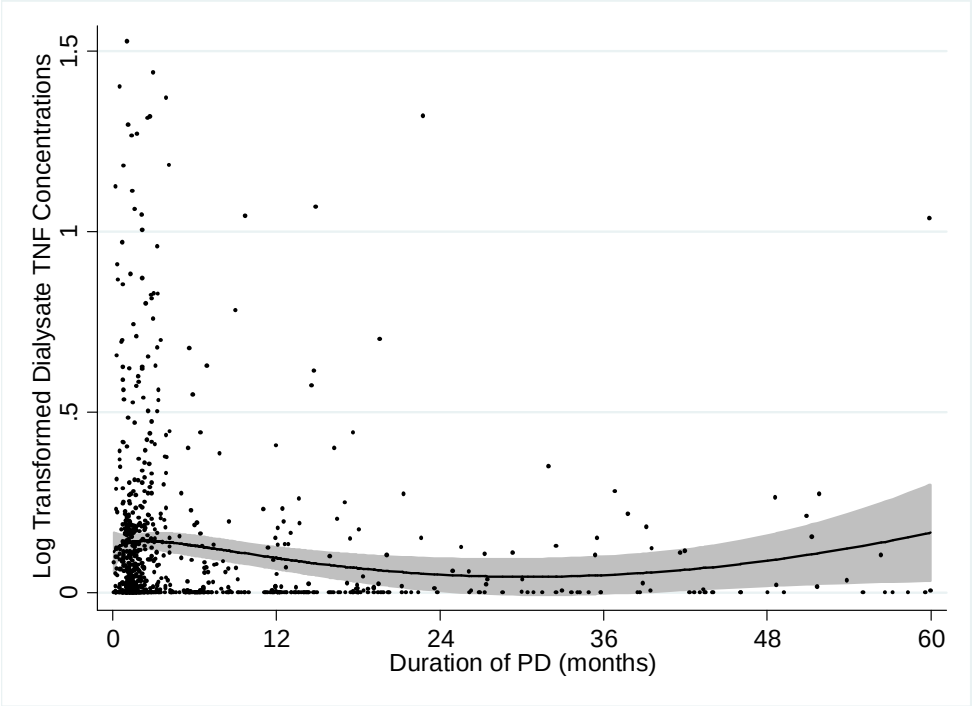


Figure 5-6: Plasma IL-6 By Duration of PD

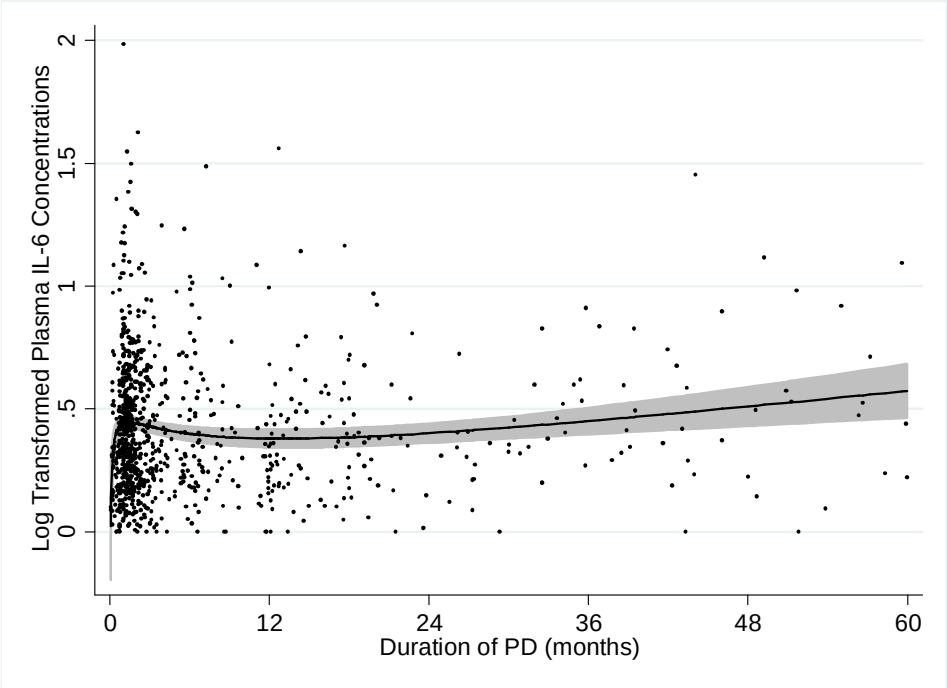


Figure 5-7: Plasma IFN-γ By Duration of PD

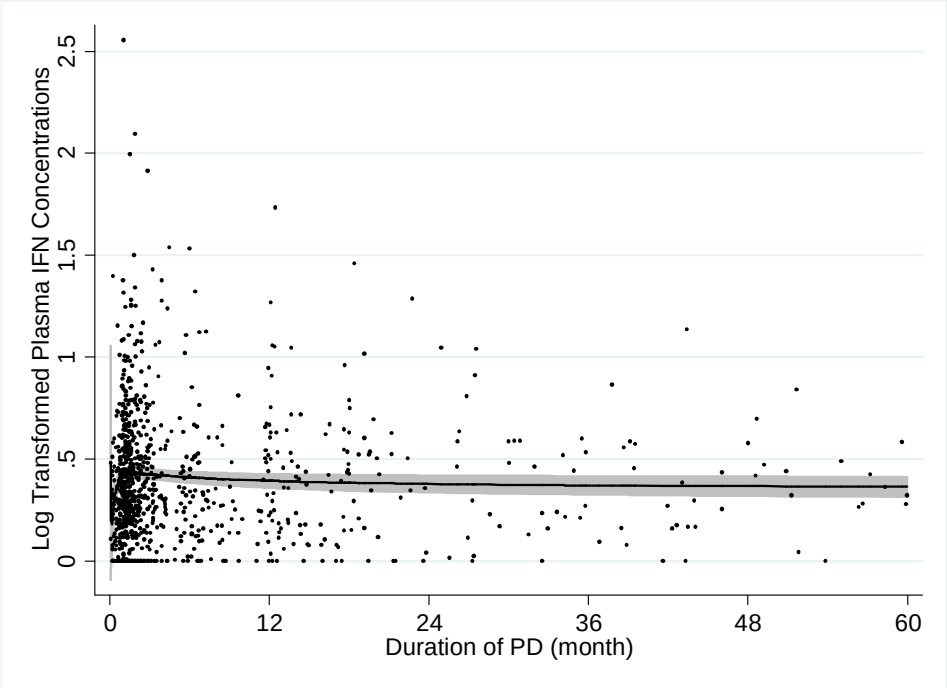


Figure 5-8: Plasma IL-1β By Duration of PD

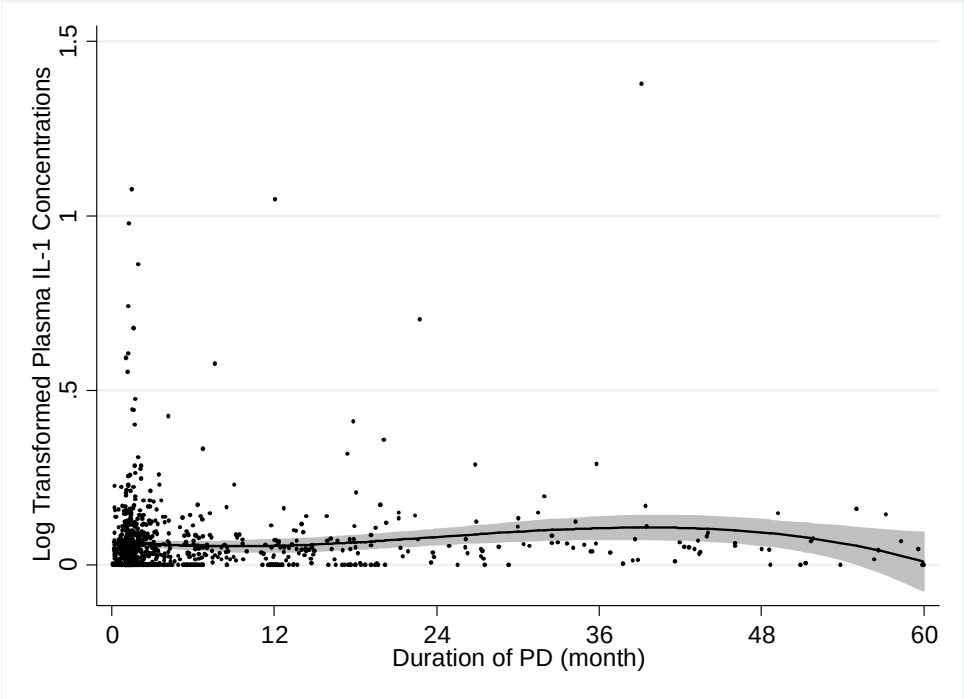
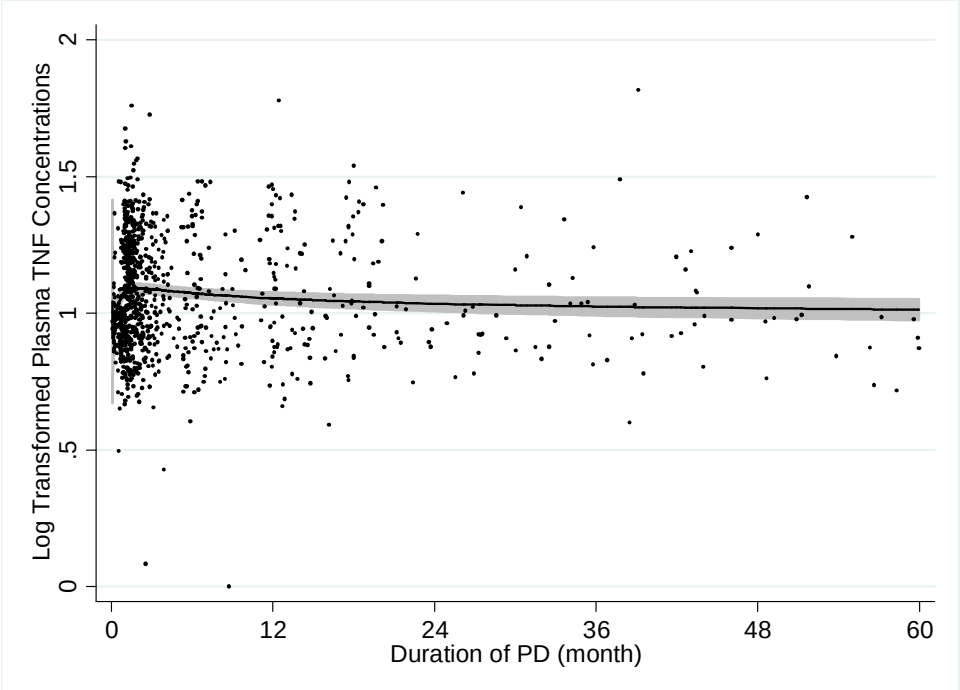


Figure 5-9: Plasma TNF-α By Duration of PD



Because of inconsistency between the correlation coefficient and the apparent flat line of the regression slope for plasma IL-1, the regression was examined in further detail with both a quartic fractional polynomial plot and a lowess regression, both of which did show some evidence of an early increase in plasma IL-1 levels.

Table 5-2: Timing in Months of Peaks and Nadirs of PSTR and Cytokine Concentrations During PD

	PSTR	Dialysate				Plasma			
	D/PCr	IL-6	TNF-α	IFN-γ	IL1-β	IL-6	TNF-α	IFN-γ	IL1-β
Initial peak	2.1	3.9	2.4	4.8	0.5	1.4	2.0	1.9	N/A
Subsequent nadir	17.3	21.9	32.4	33.9	30.7	13.0	N/A	N/A	9.1

Table 5-3: Correlations of Cytokines and PSTR with Duration of PD

		From PD Start to 2 Months		From 2 to 18 months of PD		From 18 months of PD onwards	
		Correlation Coefficient	p value	Correlation Coefficient	p value	Correlation Coefficient	p value
Dialysate	**IL-6**	**0.10**	**0.04**	**-0.13**	**0.02**	0.10	0.3
	IFN-γ	-0.08	0.10	**-0.14**	**0.009**	0.09	0.4
	IL-1β	-0.08	0.11	**-0.17**	**0.0009**	0.09	0.3
	TNF-α	-0.09	0.07	**-0.20**	**0.0001**	0.05	0.6
Plasma	**IL-6**	0.09	0.06	-0.06	0.3	0.07	0.4
	IFN-γ	**0.16**	**0.001**	-0.01	0.8	-0.09	0.4
	IL-1β	**0.10**	**0.03**	0.08	0.1	0.05	0.6
	TNF-α	**0.24**	**<0.0001**	0.02	0.6	**-0.19**	**0.04**
PSTR	**D/P Cr**	**0.19**	**0.0001**	-0.06	0.3	**0.36**	**0.0001**

To further explore the unexpected selective fall in plasma TNF-α levels, a multilevel, multivariable model examining the effect of duration of PD on plasma TNF-α was run, adjusting for the same

covariates as used in chapter 4 (see supplementary results in 11.5.2) The duration of PD had no effect (coefficient -0.0015, 95% CI -0.0095, 0.0065, p=0.71).

5.5 Discussion

This is the first large, multi-centre study examining trends with time in PSTR and inflammation, both systemic and peritoneal. Through this, we have demonstrated an increase in systemic inflammation during the first 6 to 8 weeks of PD and a fall in peritoneal inflammation from 2 to 18 months of PD. PSTR and dialysate IL-6 also had an initial rise but we could not confirm a significant fall in PSTR subsequently despite the trend apparent from the regression.

The initial sharp rise in systemic inflammation reconciles the 2 previous contradictory studies that found an increase**,** (99)and no increase,(100) in systemic IL-6 as the increase occurred after the baseline measurement in the first study, and mostly before the first measurement at one month in the second study. We have also extended the findings in Pecoits-Filho to show that the rise in systemic IL-6 is paralleled by a rise in other inflammatory markers.

If the early increase in systemic inflammation represents a true biological phenomenon, there are 2 main possible explanations – firstly, that there is a local inflammatory response to dialysate exposure which 'spills over' into the systemic circulation, and secondly that dialysate components capable of inducing an inflammatory response are absorbed into the systemic circulation.

As demonstrated in chapter 4, the peritoneal cavity during dialysis is an inflammatory environment and cytokines can have a steep diffusion gradient from the peritoneal cavity to the circulating blood. This was particularly marked for IL-6 in both our study (see Figure 4-1), and another study with a wider variety of cytokines. (183) Also, dialysate IL-6 concentrations rise during the first 2 months of PD, mirroring the changes in plasma inflammatory cytokines. Taken together, these findings support the hypothesis that peritoneal IL-6 production induces an increase in systemic inflammation during PD. Other dialysate cytokines tended not to have such a steep diffusion gradient, and the temporal changes did not match the systemic changes.

The only components of dialysate known to be absorbed in significant amounts are glucose, GDPs and the buffer, either lactate or bicarbonate. Glucose absorption during PD is associated with hyperinsulinaemia (184) and the metabolic syndrome, (185) which are themselves associated with systemic inflammation (186) although inflammation is generally considered to cause insulin resistance rather than vice versa. One small randomised study failed to find either a significant difference, or a trend to a difference, in systemic inflammation between biocompatible or standard solutions although this does not disprove the possibility of either GDPs or the buffer causing inflammation as it was a trial between different levels of GDPs and different buffers rather than a comparison against no GDPs or buffer. (187)

The rise in PSTR over the first few weeks of PD replicates previous studies, (30) and it is notable that dialysate IL-6, one of the strongest known predictors of PSTR, rises over this timescale, as does plasma IL-6 and systemic inflammation with it. The increase in PSTR is likely to be driven by intra-peritoneal IL-6, which may drive the systemic inflammation too.

We failed to show a statistically significant fall in PSTR after the initial rise, during the time period that previous data suggested there was a fall during. (31) D/P Cr levels are known to differ between centres, possibly through different effluent creatinine assays or glucose correction factors and this analysis could not take this, or differential sampling by centres, into account. This will limit the power of our study to detect significant changes in PSTR, although there was an apparent fall in the PSTR regression slope during the time period that there was also a demonstrable fall in effluent inflammatory cytokine levels. PSTR is increased, through IL-6, by peritoneal inflammation so the decrease in peritoneal inflammation provides a mechanism to explain the falls in PSTR described by Struijk et al, (31) although the correlation was quite weak.

Potential explanations for the fall in peritoneal inflammation include a loss or change in phenotype of viable inflammatory cells within the peritoneum through repeated exposure to bio-incompatible

dialysate or, if peritoneal inflammation predicts technique failure or death over the relevant timescale, informative censoring.

The longer term rise in PSTR is well documented**,** (41) and thought to be due to angiogenesis (188) but the molecular mechanisms driving this are not known. IL-6 is a strong predictor of PSTR but the mechanism behind this is not known, with possible explanations including angiogenesis, increased vascular permeability and vasodilatation. IL-6 does not induce angiogenesis directly although it might affect this through mediators such as VEGF or angiopoietin 1 and 2. (189) If IL-6 drives the long term rise in PSTR through angiogenesis, a long term rise in dialysate IL-6 would be expected, as was found in the regression but this effect was not statistically significant.

A selective long term fall in plasma TNF-α during PD has not been described previously, but the significance of this is doubtful as the duration of PD had no effect when controlled for other covariates in a multivariable regression.

The biggest limitation to this analysis is the use of cross-sectional data to infer longitudinal change. Informative censoring can occur with this technique, through particular groups having an increased risk of stopping PD and being underrepresented later on. The early changes are less likely to be affected by this, both because fewer patients will have stopped PD and because inflammation rises rather than falls with time as would be expected with informative censoring. The longer term changes will be more susceptible to informative censoring; also, there was a scarcity of data at longer time points.

Another limitation of the study is also a strength in that there was not a pre-formed hypothesis of when important changes in PSTR and inflammation occur, such as there are in other studies with pre-defined time points for sampling. This study was therefore not driven by a specific hypothesis other than that changes can occur between previously studied time points.

The multi-centre nature of the data could not be accounted for in the analysis, so centre effects remain a potential confounder. Because of these limitations none of the findings described can be regarded as definitively demonstrated but await confirmation in a study specifically designed to measure these changes.

In conclusion, we have demonstrated that there is highly likely to be a significant increase in systemic inflammation at the start of PD, possibly linked to a significant rise in dialysate IL-6, and a medium term fall in peritoneal inflammation following this that provides a mechanism for the fall in PSTR previously described.

6 The role of inflammation in Encapsulating Peritoneal Sclerosis

6.1 Summary

6.1.1 Background

EPS is an uncommon condition, strongly associated with a long duration of PD which is associated with increased fibrosis in the peritoneal membrane. The peritoneal membrane is inflamed during PD, and inflammation is often associated with fibrosis. We hypothesised that patients who subsequently develop EPS have a more inflamed peritoneal membrane during PD.

6.1.2 Methods and Materials

We performed a nested, case control study, identifying all EPS cases in the UK limb of the Global Fluid Study and matching them on centre and duration of PD with 2 to 3 controls. Dialysate and plasma samples taken during repeated peritoneal equilibration tests prior to EPS/stopping PD from cases and controls were assayed by electrochemiluminescence for IL-1β, TNF-α, IFN-γ and IL-6. Results were analysed by linear mixed models adjusted for age and time on PD.

6.1.3 Results

11 cases were matched with 26 controls. Dialysate TNF-α, 0.64 (0.23, 1.05), and IL-6, 0.79 (0.03, 1.56), were significantly higher in EPS cases, whilst IL-1β, 1.06 (-0.11, 2.23), and IFN-γ, 0.62 (-0.06, 1.29), showed a similar trend. Only IL-6 was significantly higher in the plasma, 0.42 (0.07, 0.78). Solute transport was not significantly different between cases and controls but did increase with duration of PD.

6.1.4 Discussion

The peritoneal membrane is more inflamed during PD in patients who subsequently develop EPS, a change not apparent if peritoneal solute transport is used as a surrogate measure for peritoneal inflammation.

6.2 Introduction

EPS is an uncommon but serious condition, primarily associated with a prolonged duration of PD. It is characterised by marked fibrosis 'cocooning' the gut, leading to functional impairment with malnutrition and obstruction. Histological studies have confirmed that after diagnosis there is a significant inflammatory component intra-peritoneally in EPS patients. As shown in chapter 4, PD induces intra-peritoneal inflammation during routine PD but it is not certain if this inflammatory response is greater in those patients with subsequent EPS.

It is also now clear that increased intra-peritoneal inflammation is a strong predictor of PSTR through higher levels of IL-6. Faster solute transport in patients who develop EPS is a consistent finding in most case series published (190,191) which suggests increased inflammation but neoangiogenesis could equally explain this difference. One case-control study has shown that dialysate effluent IL-6 was significantly higher in the EPS group 2 years prior to EPS diagnosis but it was not different at other time points. (192)

EPS is also associated with an increase in systemic inflammatory markers both before and after diagnosis (193) but how this relates to intra-peritoneal inflammation is unclear. As the GFS collected dialysate and plasma samples on large numbers of routine PD patients, including prevalent patients with a prolonged duration of PD, we sought to explore the roles of intra-peritoneal and systemic inflammation prior to the onset of EPS.

6.3 Methods and Materials

Study design

This is a matched, nested, case-control study. The parent study has been described in detail elsewhere (Chapter 3.1.2) but in brief, the Global Fluid Study is an international, multicentre, prospective cohort study of incident and prevalent patients commenced in 2002. Eligible patients were any PD patients over the age of 16 providing informed consent. Incident patients were defined as first data collection time point within the first 90 days of PD. Follow up was censored in December 2011. Ten centres were included in the primary analysis, and for this analysis the 5 UK centres were selected for identification of all patients who developed EPS. These cases were then assigned 2 to 3 controls who had finished PD and not developed EPS, matched on centre and duration of completed PD episode.

Data collection

All clinical data was recorded on a custom built database (PDDB). Demography was recorded and comorbidity was assessed with the validated Stoke comorbidity index. Routine blood tests, including albumin and random blood glucose, were performed locally and, if necessary, converted into the same units. All samples of dialysate and plasma from the cases and controls were assayed for TNF-α, IFN-γ, IL-1β and IL-6 by electrochemiluminescence.

PD related measurements included residual renal function, dialysis regime and dose, and peritoneal membrane function using the peritoneal equilibration test (solute transport rate: dialysate to plasma creatinine ratio (PSTR) and net UF capacity at 4 hours with 2.27% or 3.86% glucose).

Predicted dialysate concentrations

We used the 3 pore model to predict dialysate cytokine concentrations based on molecular radius as calculated from molecular weight, and assuming diffusion only. (13)

Statistical analysis

Missing data, ranging from 0 to 4.8% for different variables, were considered missing at random and complete case analysis was used. Descriptive data was compared with chi-squared tests, t-tests or Mann-Whitney U tests depending on whether variables were categorical or continuous variables, and if continuous, whether they were normally distributed or not.

Multivariable, 3-level, random intercept linear mixed models, accounting for measurements clustering within person and person clustering within case-control groups, were used to explore determinants of log-transformed cytokine levels. Normal probability distributions were checked for level 1 residuals. Because IL-1β had a highly skewed distribution, a logistic model was used with IL-1β concentrations as a binary variable (detectable/undetectable). Models included age, as it is known to affect inflammatory cytokine concentrations, and time from sample acquisition to the end of the PD episode to account for temporal changes. Further covariates were not included as models included 3 residuals and 3 covariates with a limited number of samples although interactions were tested for. Significance testing was by the Wald test and the Iterative Generalised Least Squares method was used for coefficient estimation.

MLWin 2.26 (194) was used via runmlwin for multilevel regression and StataIC 12 (StataCorp LP, College Station, TX) for the other calculations.

6.4 Results

Demographic and basic clinical details are shown in Table 6-1. The EPS group had 41 samples and the control group had 106 samples. There were no statistically significant differences in any of the measured variables although there was a statistically insignificant but potentially clinically relevant lower age, worse residual renal function and faster PSTR at the time of first sample acquisition in the EPS group.

Table 6-1: Descriptive Data for Cases and Controls

		EPS (11)	Control (26)	p value
Time Invariant	**Age**	53.6 (15.5)	63.0 (14.8)	0.091
	Male Gender	50%	57.5%	0.86
	Comorbidity (Low/Intermediate/High)	45.5/54.5/0%	32.0/48.0/20.0%	0.45
	Completed PD Episode (months)	69.0 (35.4)	69.9 (34.4)	0.95
	Number of Samples	4 (1-7)	4 (2-6)	0.58
	Reason for stopping PD			
	- **Transplant**	18%	35%	
	- **Peritonitis**	0	30%	
	- **Other technique failure**	18%	17%	
	- **Death**	0	13%	
	- **Exit site infection**	0	4%	
	- **EPS**	36%	0	
	- **UF Failure**	18%	0	
	- **Patient choice**	9%	0	
At First Sample	**Months Till PD End**	33.2 (21.8)	31.7 (18.9)	0.84
	Urine volume (mls)	501 (0-987)	838 (169-1432)	0.27
	Icodextrin Usage	45.5%	28.0%	0.31
	Dialysate Glucose Exposure	165.0 (74.9)	138.9 (42.6)	0.36
	APD usage	18.2%	20.0%	0.90
	Dialysate IL-6	10.67 (8.04-21.53)	6.05 (1.54-14.89)	0.18
	Plasma IL-6	0.95 (0.85-1.76)	1.30 (0.71-2.63)	0.61
	Serum Albumin	37.3 (5.0)	36.8 (5.8)	0.91
	D/P Cr	0.81 (0.15)	0.72 (0.16)	0.13
	Blood Pressure - Systolic/Diastolic	142/83 (24/16)	146/83 (26/11)	0.64/0.77

Figures are proportions, mean (SD) or median (IQR) depending on variable type and skewness.

Determinants of dialysate and plasma cytokine concentrations, as found with multilevel multivariable models, are shown in Table 6-2. All dialysate cytokine concentrations tended to be

higher in EPS cases but the difference was only statistically significant for IL-6 and TNF-α. Of the plasma cytokines, only IL-6 was significantly higher in EPS cases although the average difference was only 0.42 $\log_{10}$ concentrations, compared to 0.79 $\log_{10}$ concentrations in dialysate IL-6. An increase with time was apparent for both dialysate and plasma IL-6 as well as for plasma IFN-γ. PSTR rose with time but PSTR was not statistically significantly different between EPS cases and controls (Figure 6-1). If the D/P Cr at the time of the last sample was compared, there was still no significant difference in PSTR between groups (EPS 0.820, Control 0.818, p=0.98). Age was associated with higher plasma cytokines except for IL-1β but out of the dialysate cytokines it was only associated with TNF-α. There were no significant interactions between EPS status, age and time to PD finish although the power to detect them would have been weak.

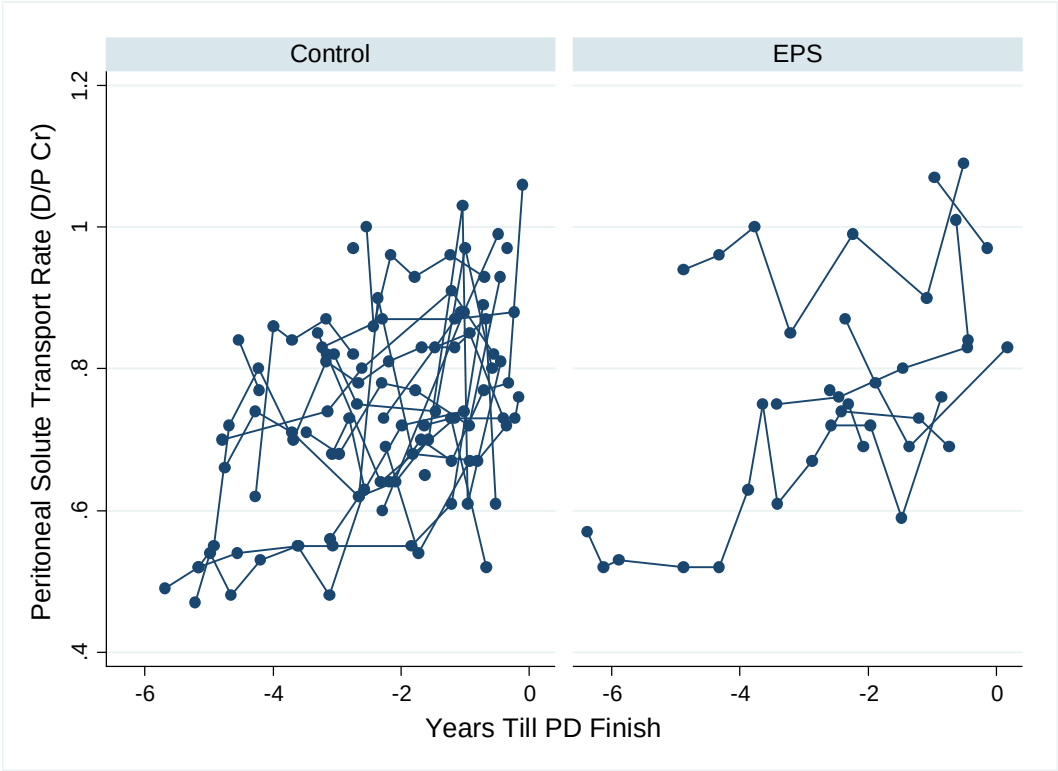
Table 6-2: Determinants of Inflammatory Cytokine Levels by EPS Status

Dependent Variable		EPS		Age		Time Till PD End	
		Coefficient (95% CI)	p value	Coefficient (95% CI)	p value	Coefficient (95% CI)	p value
Dialysate	IL-6	0.79 (0.03, 1.56)*	0.043	0.009 (-0.014, 0.033)	0.43	0.27 (0.13, 0.42)*	<0.001
	IL-1β	1.06 (-0.11, 2.23)	0.075	0.022 (-0.012, 0.056)	0.20	0.19 (-0.08, 0.47)	0.17
	IFN-γ	0.62 (-0.06, 1.29)	0.073	0.016 (-0.005, 0.036)	0.14	0.085 (-0.045, 0.215)	0.20
	TNF-α	0.64 (0.23, 1.05)*	0.002	0.019 (0.007, 0.031)*	0.001	0.048 (-0.026, 0.123)	0.20
Plasma	IL-6	0.42 (0.07, 0.78)*	0.020	0.016 (0.005, 0.026)*	0.003	0.13 (0.05, 0.21)*	0.001
	IL-1β	0.66 (-0.65, 1.97)	0.33	-0.023 (-0.064, 0.017)	0.26	-0.21 (-0.55, 0.13)	0.23
	IFN-γ	-0.30 (-0.69, 0.09)	0.14	0.014 (0.001, 0.027)*	0.036	0.12 (0.02, 0.22)*	0.017
	TNF-α	0.13 (-0.13, 0.39)	0.31	0.010 (0.002, 0.017)*	0.011	0.45 (-0.007, 0.098)	0.090
Solute Transport	D/P Cr	0.024 (-0.054, 0.102)	0.55	-0.0017 (-0.0039, 0.0006)	0.14	0.035 (0.023, 0.047) *	<0.001

*p<0.05. Results from models with continuous dependent variables (log transformed if cytokine) except for dialysate and plasma IL-1 models which were logistic models for detectable vs undetectable.

34.4% of dialysate samples had a concentration of TNF-α greater than predicted by diffusion according to the 3 pore model, assuming plasma TNF-α exists as a homotrimer. If plasma TNF-α was assumed to be monomeric the proportion was 31.9%.

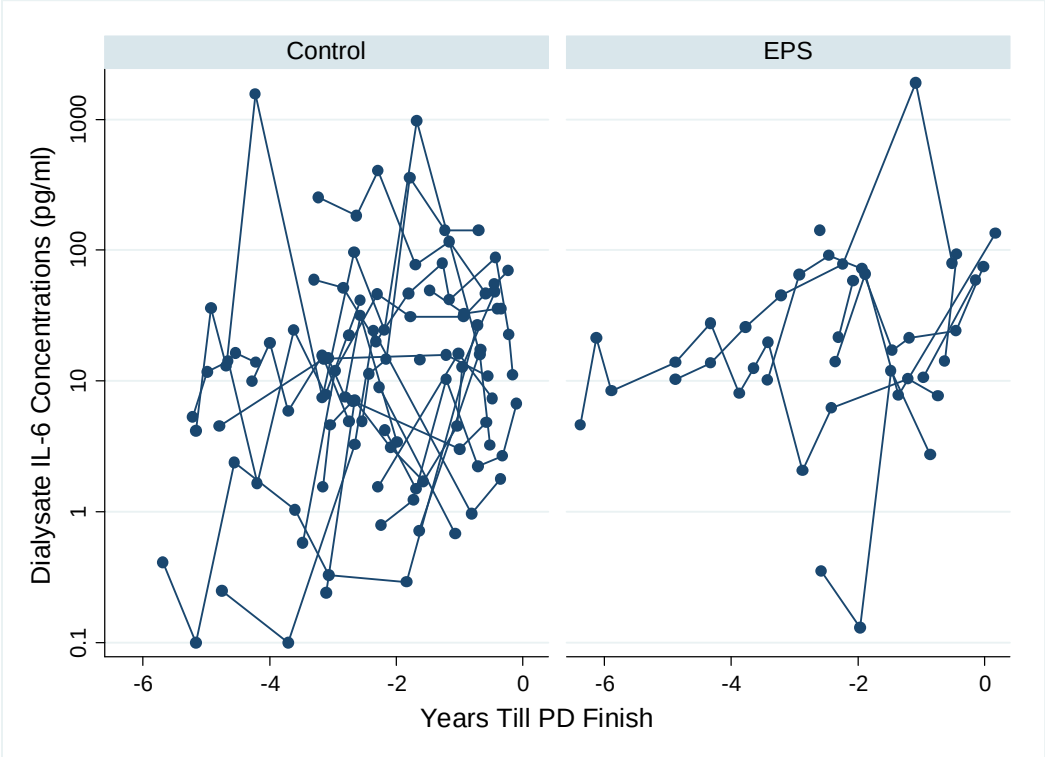
Figure 6-1: Peritoneal Solute Transport Rate With Time to PD Finish By EPS Status



Circles represent individual measurements, with lines identifying different patients

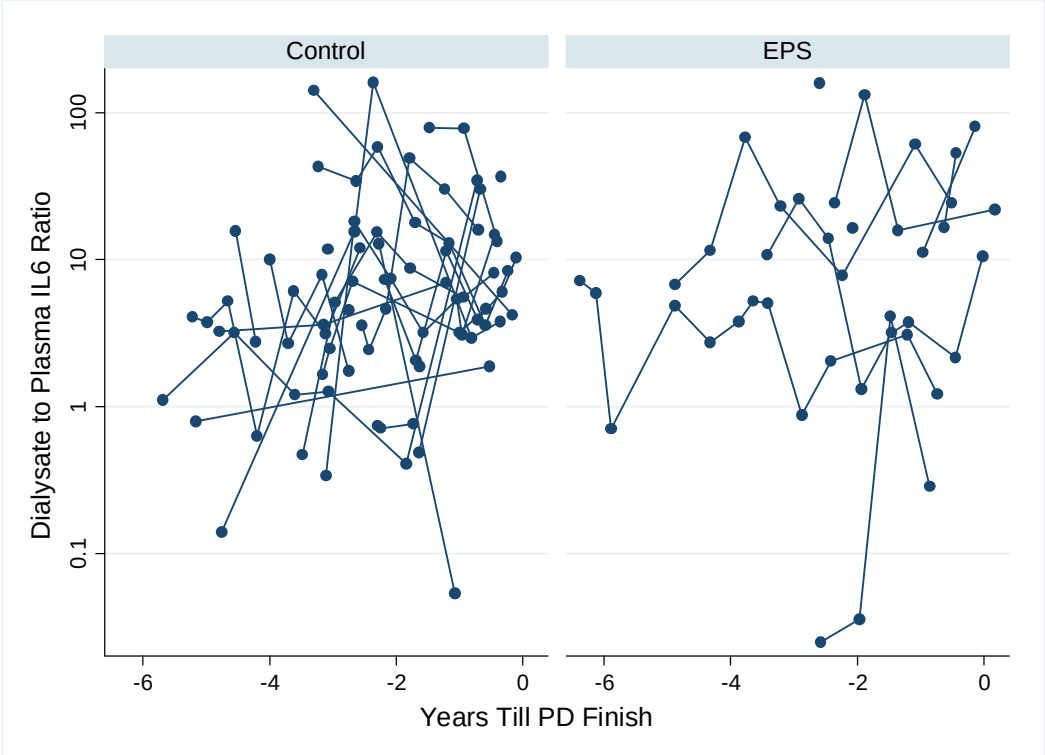
The increase in dialysate IL-6 is demonstrated in Figure 6-2 , but there is no apparent change in the ratio of dialysate to plasma IL-6 (Figure 6-3). A univariable, multilevel regression model for dialysate to plasma IL-6 ratios confirmed that time had no effect (coefficient 0.98, 95% CI -2.65 to 4.60, p=0.6). The dialysate TNF-α results are demonstrated in Figure 6-4.

Figure 6-2: Dialysate IL-6 With Time to PD Finish By EPS Status



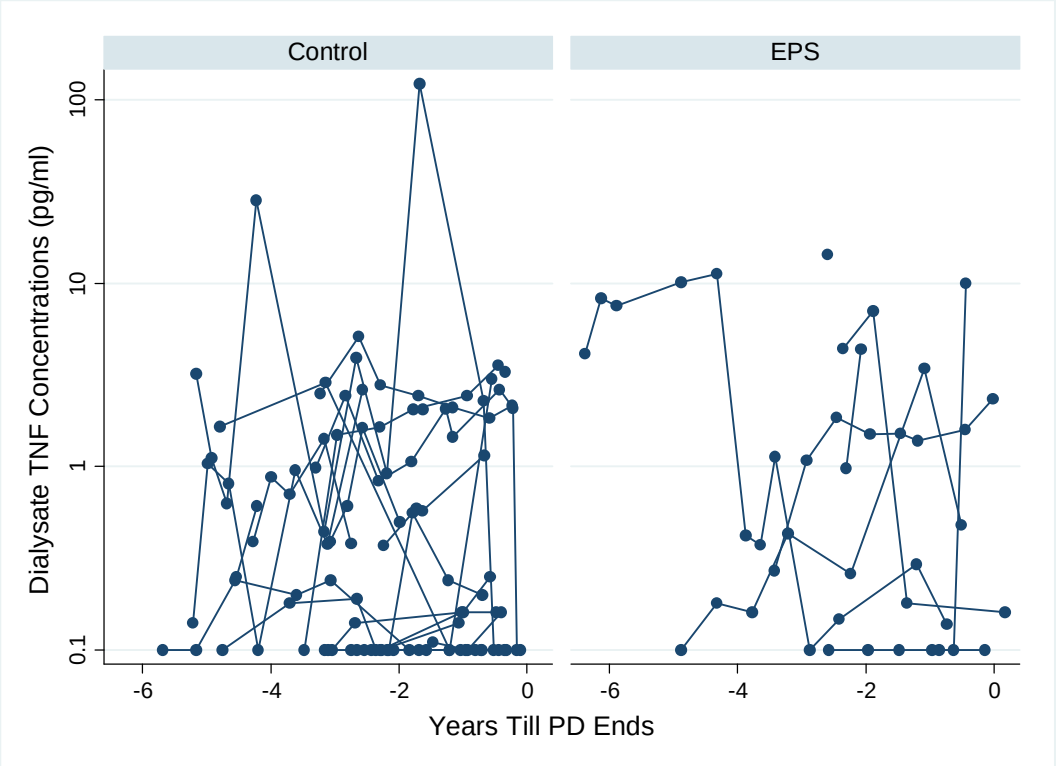
Circles represent individual measurements, with lines identifying different patients

Figure 6-3: Dialysate to Plasma IL-6 Ratio With Time to PD Finish By EPS Status



Circles represent individual measurements, with lines identifying different patients

Figure 6-4: Dialysate TNF-α With Time to PD Finish By EPS Status



Circles represent individual measurements, with lines identifying different patientsAll values are TNF concentration +0.1 to allow a logarithmic scale (i.e. 0.1 = 0)

6.5 Discussion

We have demonstrated for the first time that intra-peritoneal inflammation, compared to controls who do not develop EPS, is increased for several years prior to EPS and this difference in intra-peritoneal inflammation is more readily apparent than the associated difference in PSTR. There is also an increase in plasma IL-6, although the magnitude of this difference is less marked, and none of the other plasma cytokines are elevated.

One of the unexpected findings was the lack of difference in solute transport which is frequently reported as a risk for EPS. Some of the other studies of solute transport in EPS have not been able to accurately match for time on PD because the strong association of EPS with long periods of PD makes finding controls with solute transport measured after the same duration of PD difficult (190,191) whilst another study found no difference in PSTR between EPS and UF failure.(195) Our study has matched very effectively for time on PD such that there is no significant difference in the total duration of PD, or in PD duration at the time of first sampling. The importance of this is highlighted by the significant independent effect that time had on solute transport in our study. Chapter 5 compared 9 EPS cases, matched such that the control patients average time on PD was only an average of 3.7 months less, found no difference in solute transport until the last year prior to EPS/PD finishing (although 6 patients from that study are included in this one). This suggests that PSTR is not a good method for identifying patients who subsequently develop EPS.

We have replicated a previous finding of a raised dialysate effluent IL-6 in patients who subsequently develop EPS, (192) but extended the finding as that study only found a difference at 2 years prior to the development of EPS and not at 1 or 3 years prior. Our study had more samples, but also used all samples available in a single model, demonstrating that dialysate IL-6 concentrations are higher in EPS patients at all time points covered (from 6 years prior to stopping PD up to PD cessation). As

samples are only available from one EPS patient between 5 and 6 years prior to stopping PD, the certainty in the conclusions fall at this point, but the separation between EPS and control patients did not change over the duration of the study period (i.e. no significant interaction between EPS status and time to PD cessation).

We have also shown for the first time that dialysate effluent TNF-α is significantly higher in patients who subsequently develop EPS and appears to be more promising as a biomarker for EPS risk than IL-6. As increased fibrosis manifesting as decreased osmotic conductance to glucose increases the risk of EPS (195,196) any biomarker for EPS is likely to be associated with fibrosis. TNF-α has previously been considered an antifibrotic molecule based on in vitro work, but there is some preliminary evidence in rheumatological conditions suggesting that TNF-α inhibitors may reduce fibrosis. (197) Whether TNF-α is directly implicated may depend on whether the fibrosis associated with EPS is driven by inflammation or through another mechanism.

Any dialysate biomarker predictive of EPS is likely to reflect local pathophysiology and therefore will be produced locally however previous studies of dialysate TNF-α have found low levels compatible with its presence being due to diffusion from the systemic circulation. (103,198) These studies have used a molecular weight based on TNF-α being a monomer to calculate the predicted dialysate concentration due to diffusion but TNF-α is reported to predominantly exist as a homotrimer. We used predictions based on both a monomeric and a homotrimer form of TNF-α and found little difference between the two but we did find that more samples than reported previously had evidence of local production. Furthermore, TNF-α has previously been shown to significantly correlate with other intra-peritoneal cytokine levels for which there was clear evidence of local production.(198) This would be compatible with intra-peritoneal TNF-α, as part of an inflammatory cascade, inducing fibrosis and thereby predisposing patients to EPS. TNF-α has previously been linked with anti-fibrotic effects based

primarily on in vitro data, however there is some clinical data based on the effects of anti-TNF-α agents in rheumatological diseases suggesting that it might have a pro-fibrotic role. (197)

Both IFN-γ and IL-1β showed a trend towards higher levels in the EPS group. Neither of these findings quite met the pre-set p value for statistical significance of 0.05, but the findings were certainly supportive of the hypothesis that patients who develop EPS have a more inflamed peritoneal membrane during PD.

We have also examined changes in plasma levels of inflammatory cytokines preceding EPS, demonstrating an isolated increase in plasma IL-6. Previous studies have only examined CRP before the diagnosis of EPS, showing either no difference (199) or a difference one year prior to diagnosis (193). We have therefore extended this by showing changes evident systemically well before the diagnosis of EPS is made, but also provided a potential mechanism for this. There is a clear diffusion gradient from dialysate to plasma for IL-6 that is not present for most inflammatory cytokines suggesting that clearance of IL-6 from the peritoneum contributes to systemic levels. Further suggestive evidence comes from the greater difference in dialysate compared to plasma IL-6 concentrations between EPS cases and controls as, if this theory is correct, a doubling in dialysate concentrations would be unlikely to lead to a doubling in plasma concentrations.

Limitations of this study include a relatively small number of EPS cases, although it is an uncommon condition so this still represents the largest collection of dialysate effluent samples pre-diagnosis. As an observational study, cause and effect cannot be proven so it is not definite that inflammation is pathophysiologically involved in driving EPS.

7 Changes in the peritoneal membrane preceding Encapsulating Peritoneal Sclerosis

See also Appendix C – Note on Statistical Tests in Chapter 7

7.1 Summary

Encapsulating peritoneal sclerosis (EPS) is a serious condition occurring with increasing frequency the longer the duration of peritoneal dialysis. We report the longitudinal changes in peritoneal membrane function of patients who develop EPS compared with controls who had completed their dialysis episode. All patients starting peritoneal dialysis since 1990 with an unequivocal diagnosis of EPS in our unit were identified, and each matched for dialysis duration and age with four controls. All prospectively collected clinical measures were retrieved and compared. The dialysate/plasma creatinine ratio increased with time in both groups but was only significantly higher in EPS patients at the time of stopping dialysis (0.89 vs 0.78, p=0.007), whereas the ultrafiltration capacity was significantly worse from at least 2 years prior to stopping dialysis, diverging further till dialysis finished (345mls vs 137mls, p=0.006) suggesting reduced osmotic conductance. Both the glucose exposure rate for the 5 years preceding stopping dialysis and the Icodextrin exposure was significantly higher, with a worse residual renal function, in the EPS group, but there was no significant difference in peritonitis rates. 24 hour peritoneal protein clearance was not significantly different in EPS cases, possibly due to a greater fibrous matrix. This study shows that regular peritoneal membrane function tests can identify most patients at high risk of developing EPS.

7.2 Introduction

Encapsulating peritoneal sclerosis (EPS) is an increasingly recognised complication of peritoneal dialysis(PD), characterised by development of a diffuse 'cocoon' over the small bowel causing gastrointestinal symptoms and bowel obstruction, leading to weight loss, under-nutrition and in some cases death. The incidence increases with time on PD, ranging from 0% during the first 3 years to 5.9% for patients on PD between 8 and 10 years in Japan, (200) and from 0% during the first year to 8% for patients on PD for 5 or more years in Scotland.(168) Several treatment-related risk factors have been suggested including severe peritonitis, glucose exposure, lack of residual renal function and the development of ultrafiltration failure associated with high rates of membrane solute transport. Paradoxically, discontinuation of PD appears to be a trigger factor for developing the condition resulting in a therapeutic dilemma for the clinician as to when and if patients should be switched to haemodialysis (HD). Although EPS is rarely seen in patients not exposed to PD, other precipitants such as cirrhosis with ascites, post-surgical, peritoneal shunts, autoimmune conditions and intraperitoneal chemotherapy (201,202) are described and the underlying pathophysiology remains obscure. This is compounded by the lack of a good animal model, the rarity of the disease and the lack of stringent diagnostic criteria.

The ability to identify patients at high risk, thereby prompting consideration of switching to HD or transplantation might be facilitated by identifying changes in peritoneal membrane function that precede or predispose to the development of EPS. It is well established that time on PD is associated with increases in peritoneal solute transport rate (PSTR) associated with a fall in the ultrafiltration (UF) capacity of the membrane. In a proportion of patients there is uncoupling of these two processes due to a decrease in the osmotic conductance of the membrane that may well reflect the progressive fibrosis associated with longer time on treatment. These changes in membrane function are associated with more rapid loss in residual renal function and higher glucose exposure, which may contribute to further fibrosis and subsequent EPS. To date, most studies of EPS patients have

reported only the results of the most recent peritoneal membrane function tests prior to diagnosis. We report the first controlled longitudinal cohort study of peritoneal membrane function with associated detailed clinical measurement including RRF and peritoneal glucose exposure in patients who subsequently develop definite EPS.

7.3 Methods

For further statistical comments, see Appendix C – Note on Statistical Tests in Chapter 7

7.3.1 Study Design

This was a nested case control study. We identified all patients from the well-defined Stoke PD patient cohort with probable EPS, and reviewed their clinical records including nursing documentation and radiological reports. To avoid some of the uncertainty surrounding early EPS, only patients with definite EPS (surgically or radiologically confirmed peritoneal membrane thickening and cocooning, in conjunction with weight loss and features of bowel obstruction) were selected. Two patients in whom EPS was associated with persisting poorly controlled peritonitis were excluded. Each of these patients was then matched by time on PD, and age where possible, with 4 controls from the same cohort by identifying adjacent patients from the whole database who had completed their treatment episode on PD, neither deliberately including nor excluding controls with UF failure. 4 controls were selected to maximise statistical power whilst retaining efficiency. (203)

7.3.2 Prospective collection of routine clinical measurements

Baseline demography and PD measurements for cases and controls were retrieved from the database, along with the total number of peritonitis episodes per patient, and comorbidity, as assessed by the validated Stoke comorbidity index which categorises patients into low (score 0), intermediate (score 1-2), and high (score >2) risk groups.

Routine 6 monthly measurements included residual renal function, dialysis regime and dose, and peritoneal membrane function using the peritoneal equilibration test (solute transport rate: dialysate to plasma creatinine ratio (D/P Cr) at 4 hours and net UF capacity with 2.27% glucose) were obtained from the database. The 'overfill' included in the bags was not subtracted from the 4 hour ultrafiltration capacity, but consistently measures 200mls in our unit. Adequacy was expressed as

weekly Kt/Vurea for the peritoneal component, whereas the urine volume and mean weekly urea and creatinine clearance (litres/week/1.73m^2) were used to determine residual renal function.

Glucose exposure was calculated by summing the grammes of anhydrous glucose within the daily dialysate used for each regime the patient had, multiplying this by the number of days they were on that regime, and combining the results for each regime for the annual total glucose exposure (grammes) of the peritoneal membrane. Icodextrin use was recorded as a categorical value for whether it was used at any point in the year in question.

Peritoneal protein losses were measured from the 24 hour total dialysate collection assayed by the Biuret method. A validated correction factor (204) was then used in the equation: PCl = 24 hour dialysate protein loss/(serum albumin/0.4783), with PCl representing the peritoneal protein clearance expressed as mls of plasma per day. Regular dialysate protein concentrations were measured from 1999, providing data on 8 EPS cases (46 samples) and 28 controls (107 samples) within the last 4 years of PD.

7.3.3 Statistical Analysis

To compare the evolution of clinical measurements prior to the study endpoint (diagnosis of EPS for cases and completion of PD episode for controls), measurements were aligned from the point of stopping PD backwards, such that cessation of PD counted as time 0, with one year prior to this counting as -1, and so on until year -8. This method was used for UF capacity, solute transport and glucose exposure and the data is all expressed as the yearly mean value for each group. As measurements were taken 6 monthly, this usually represents the mean of two readings per patient.

As residual renal function declines with time on treatment, the measurements of urine volume were aligned from starting PD forward to year +8. Comparisons between EPS cases and controls were made with all values obtained within yearly intervals, and all values from the first 4 years of PD.

Between-group comparisons used the 2-tailed unpaired t-test, the Mann-Whitney U test or the chi-squared test depending on the data type and distribution. The paired t-test was used for longitudinal comparisons, using all measurements between 4.5 and 5.5 years before PD finished and comparing them with readings taken in the last year of PD. These figures were chosen to include measurements from as many patients as possible whilst maintaining a meaningful difference in time.

The risk of developing EPS with time on treatment for the whole PD cohort was determined from a Kaplan Meier survival analysis of all patients commencing PD after 1/1/1990, with the definition of EPS for the study extended to include cases associated with severe and prolonged peritonitis. A linear mixed model was used to examine the PCl data as well as t-tests. These analyses were with SPSS v17.

7.4 Results

9 patients with definite EPS were identified from a total cohort of 692 patients treated with peritoneal dialysis since 1990. The diagnosis of EPS was confirmed surgically in 5 out of 9 patients, and radiologically in 7 out of 9, (Table 7-1). 3 patients were on PD at the time of diagnosis, while the other 6 were on haemodialysis, a mean of 7.3 months after stopping PD (range 1-18 months). Constipation was unusual, while diarrhoea occurred in only 3 out of 9. Frank obstruction was only diagnosed formally in 4 out of 9 although vomiting occurred in 6 out of 9.

EPS free survival is demonstrated in Figure 7-1. The 2 early cases of EPS (6 and 23 months) were both related to severe, prolonged peritonitis episodes. Of the EPS not associated with severe and prolonged peritonitis, there were no early cases, with the first case occurring at 32 months. The risk rose substantially after five years such that the probability of EPS free survival after 93 months on PD was 0.85. The confidence intervals for this are wide with small numbers of patients at risk but they follow a pattern of an increasing gradient with time in the Kaplan-Meier plot.

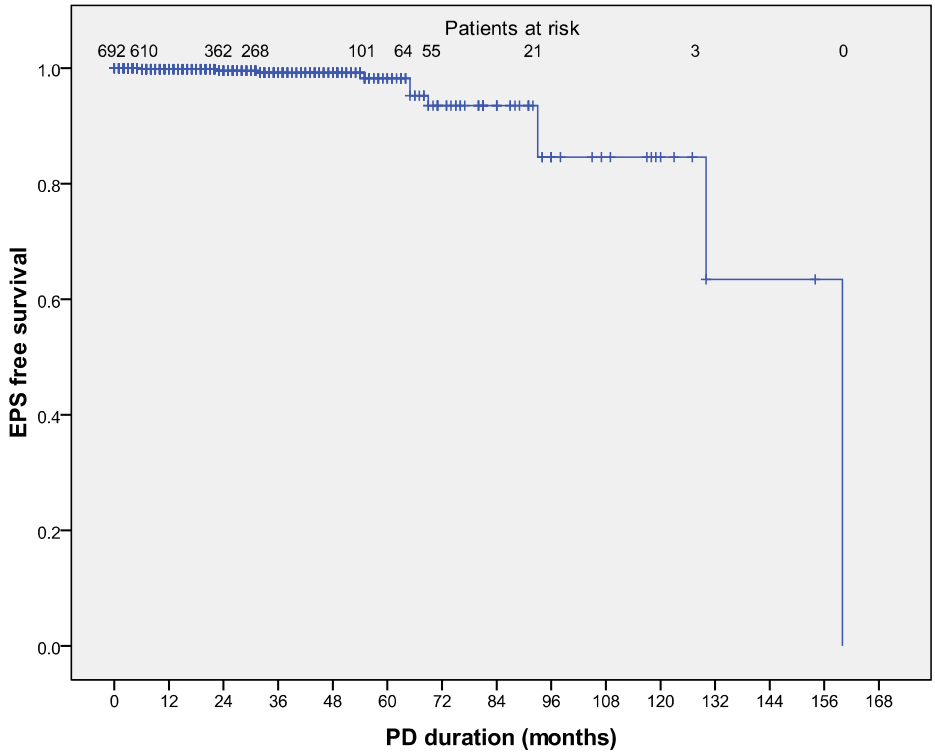
The demographics of the cases and 36 matched controls are shown in Table 7-2 with no significant difference between the groups. One EPS case had 2 significant sessions of PD which, whilst separated by a 9 year period, were combined into one for the analysis. The cohorts were reasonably matched in time with a median date of stopping PD of 26/3/04 in EPS cases and 9/4/02 in the controls. Predictably, the outcome of the two groups was significantly different.

The mean number of peritonitis episodes was greater in the control group, although this did not reach statistical significance (1.78 vs 4.19, p=0.061). 2 EPS cases had no peritonitis at all, only one EPS case had their catheter temporarily removed for peritonitis and only 2 of the EPS cases had peritonitis within the 6 months preceding stopping PD.

Table 7-1: Clinical Features of EPS Cases

EPS case	Age at diagnosis	Gender	Cumulative months on PD	Diagnosis days post PD	Days survival post diagnosis	Cause of death	Reason for stopping PD	Abdominal pain	Ascites	Vomiting	Weight loss	Radiologically confirmed	Surgically confirmed	Surgical treatment	Tamoxifen treatment	Steroid treatment
1	45.8	F	161	0	7	Multi-organ failure post operatively	EPS	Y	Y	Y	N	Y	Y	Y	N	N
2	34.6	F	130	0	33	Multi-organ failure	Peritonitis	Y	Y	Y	Y	N	Y	N	N	N
3	39.5	M	93	36	>729	Alive	EPS	Y	Y	Y	Y	Y	Y	Y	Y	Y
4	71.5	M	65	0	2	Bowel obstruction	EPS/died	Y	N	Y	N	Y	N	N	N	N
5	54.7	M	69	101	2	Sudden death after vomiting++	UF failure	Y	Y	Y	Y	N	Y	N	N	N
6	65.6	M	65	560	120	Malnutrition, withdrawal of HD	Drainage problems	Y	Y	Y	Y	Y	N	N	Y	N
7	44.3	F	55	320	221	Sudden cardiac death	UF failure	N	Y	N	Y	Y	N	N	Y	N
8	26.8	F	93	47	>344	Alive	UF failure	Y	Y	Y	Y	Y	Y	Y	N	Y
9	60.0	M	32	296	48	Malnutrition	Patient choice	Y	Y	Y	Y	Y	N	N	Y	Y

Figure 7-1: Kaplan-Meier Plot of EPS Free Survival



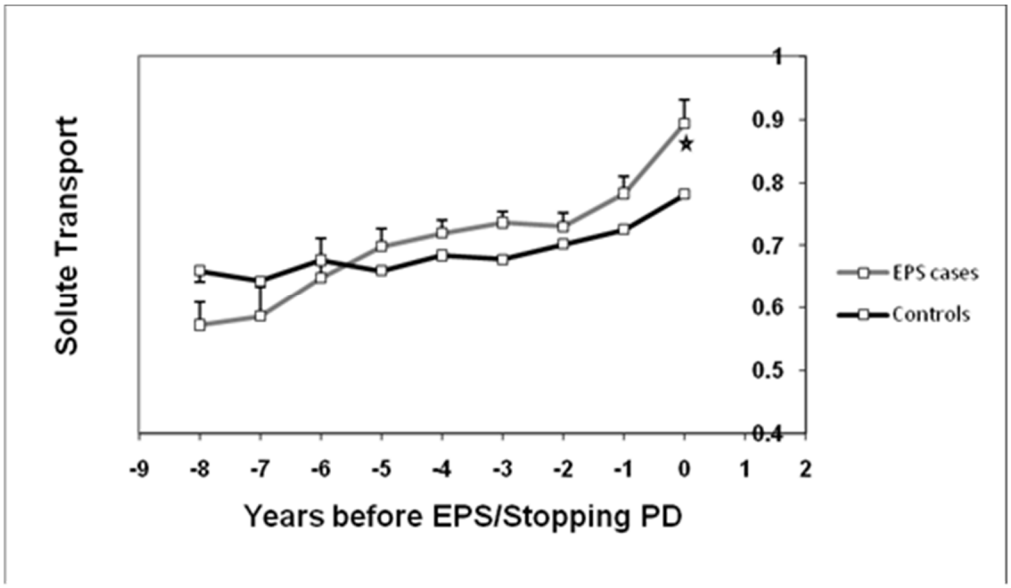
The probability of remaining free of EPS with duration of time on PD. This includes 2 early cases associated with severe, prolonged peritonitis. The number of patients at risk at the time of each case of EPS is shown at the top.

Table 7-2: Demographics of EPS Cases and Controls

	EPS (9 patients)	Controls (36 patients)	p value
Duration of PD in months (months; mean)	84.7	81.0	0.27
Age at PD cessation (years; mean)	48.8	54.7	0.32
Male Gender (%)	55.6	50	0.77
Ethnicity			
- **White European** (%)	88.9	97.2	0.28
- **South Asian** (%)	11.1	2.8	
Stoke comorbidity index (mean)	0.78	0.78	0.98
- **Low** (%)	55.6	50.0	0.76
- **Intermediate** (%)	44.4	44.4	
- **High** (%)	0	5.6	
Diabetic (%)	0	16.7	0.19
Adequacy at treatment start			
- **Renal Kt/V** (median)	0.19	0.98	0.24
- **Renal Cr clearance** (median)	9.86	48.4	0.27
- **Peritoneal Kt/V** (mean)	1.60	1.60	0.61
PET test at treatment start			
- **D/P Cr** (mean)	0.71	0.66	0.18
- **UF capacity** (mean)	402	423	0.82
Modality immediately post PD			
- **Haemodialysis** (%)	88.9	30.6	
- **Transplant** (%)	0	33.3	
- **Died** (%)	11.1	36.1	

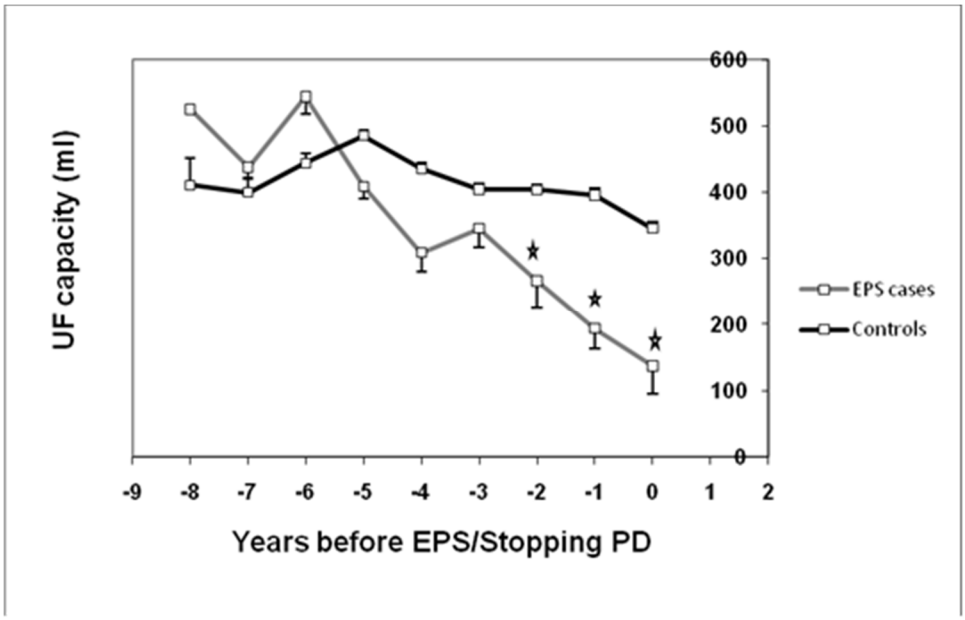
Both EPS and control groups demonstrated a gradual rise in solute transport rate with time on treatment (p=0.003 and 0.001 respectively), (Figure 7-2). The change with time of UF capacity in the EPS group (Figure 7-3) was significant (p=0.02) but not in the control group (p=0.34). Only 2 of the control group of patients had a consistently low UF capacity suggestive of UF failure.

Figure 7-2: Change in Membrane Solute Transport with Time on PD



Solute transport is measured as the mean annual D/P Creatinine measured at 4 hours in standard peritoneal equilibration tests with standard error. The star indicates a difference between groups with p=0.007.

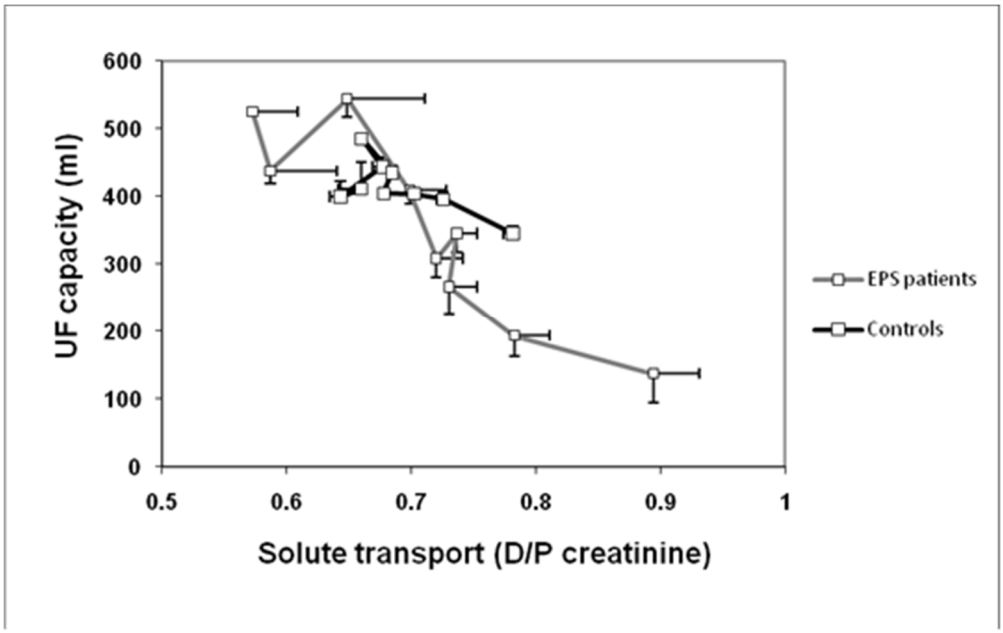
Figure 7-3: Change in Ultrafiltration Capacity with Time on PD



The UF capacity is the annual mean measured by a 4 hour peritoneal equilibration test with 2.27% glucose, including approximately 200mls overfill. Bars represent standard error. The stars indicate a difference between groups with $p<0.05$, un-paired t-test.

When D/P Cr and UF capacity are plotted together (Figure 7-4), the decline in UF capacity with increasing D/P Cr is disproportionately worse for the EPS group such that, for the same D/P Cr ratio in both groups, there is a statistically significant difference in UF (345mls at 0.781 for control group vs 194mls at 0.784 for EPS group, p=0.015).

Figure 7-4: Change in Membrane Solute Transport with Change in Ultrafiltration

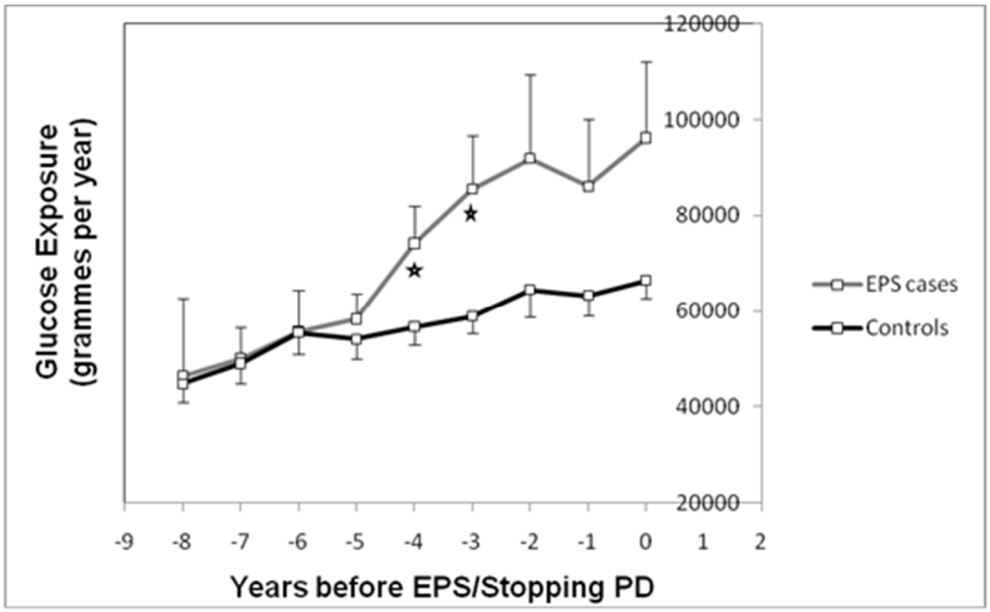


Membrane solute transport and ultrafiltration are measured as the annual mean of measurements of UF capacity and D/P Creatinine in a 4 hour PET with 2.27% glucose, including approximately 200mls overfill. Bars represent standard error.

5 EPS cases had been on APD, giving a mean duration on APD usage of 20.2 months within EPS cases, and 19 controls had been on APD, giving a mean duration of 13.1 months within controls. This difference was not significant (p=0.35). The mean annual glucose exposure rates (Figure 7-5) were not statistically significantly different for the last 3 readings due to an increase in variance but the mean values still showed a clear difference. The last year's glucose exposure was 66,494 and 96,241 grammes per year in control and EPS groups respectively; 4 two litre 1.36% or 2.27% exchanges per day correspond to a yearly glucose exposure of 39,712 or 66,284 grammes per year respectively.

Icodextrin use was greater in the EPS cases, but the difference was only significant for between 1 and 2 years prior to stopping PD (7/9 vs 12/36, p=0.016).

Figure 7-5: Glucose Exposure with Time on PD

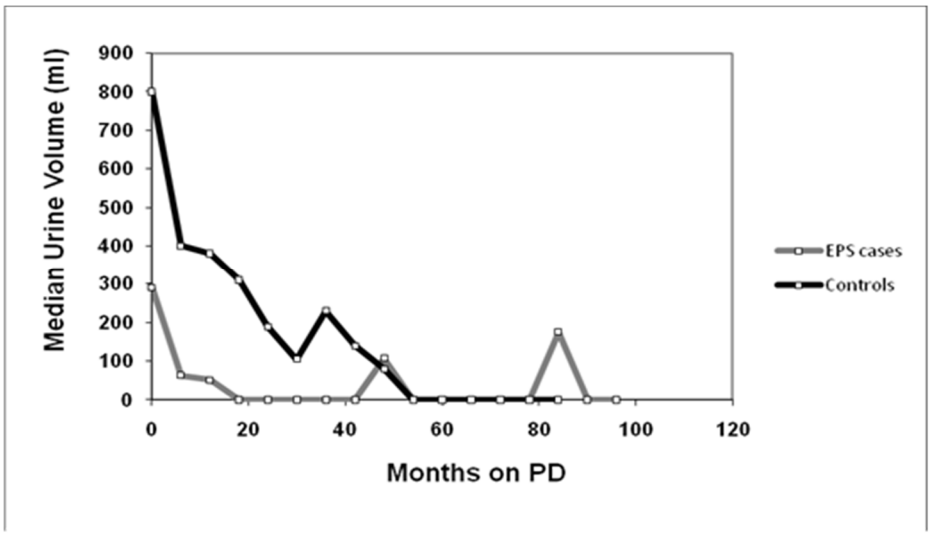


Glucose exposure is measured as the mean of the total number of grammes of glucose in the dialysate used by each patient in that year. Bars represent standard error. The stars indicate a difference between groups with $p<0.05$, un-paired t-test.

Over the first 4 years of PD residual renal function was significantly better preserved in controls with a mean urine volume for EPS 339.6mls vs controls 572mls, p=0.001 (Figure 7-6). Peritoneal protein clearances (PCl) were not significantly lower in the EPS cases than the controls by independent samples t-test (Figure 7-7). The EPS group did have a lower PCl than controls in a linear mixed model with EPS/controls and time to PD finish as predictor variables using fixed effects but the model improved and the difference became insignificant when a random effect was added for EPS/controls or intercept. Although there were insufficient samples to make meaningful comparisons on a time

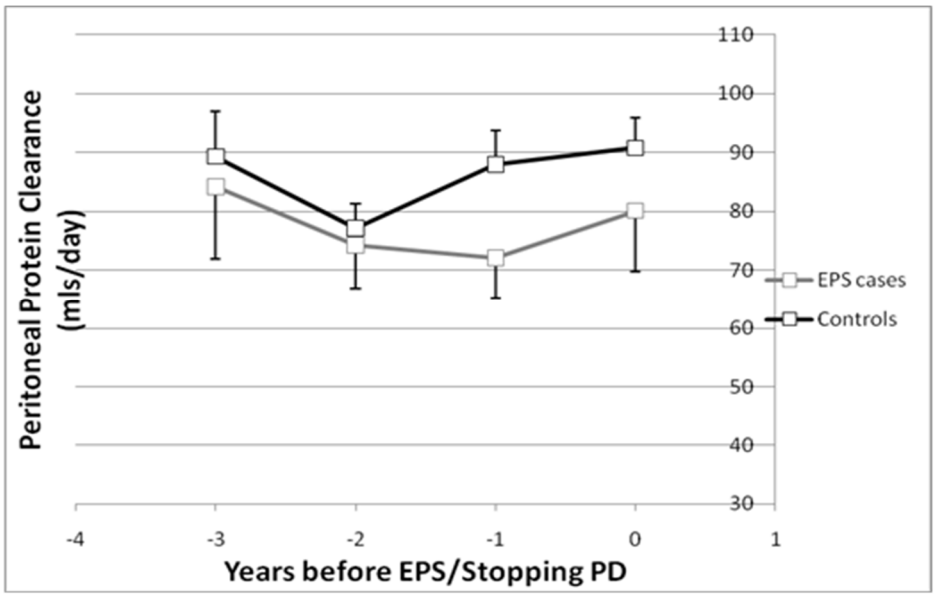
matched basis, there was no significant longitudinal change from the last year of PD to between 3 and 4 years before stopping PD (94.50 vs 84.99 mls plasma per day, p=0.46).

Figure 7-6: Residual Renal Function with Time on PD



The residual renal function is measured as the median of the urine volume within each group assessed every 6 months from the start of PD.

Figure 7-7: Peritoneal Protein Clearance with Time on PD



Peritoneal protein clearance is measured in mls of plasma per day and the bars represent standard errors. There were no significant differences between the groups when applying mixed linear modelling.

7.5 Discussion

This is the first controlled analysis describing the longitudinal changes of a number of treatment related measures of PD patients developing EPS. The characteristics of patients destined to develop EPS were early loss of residual renal function, and increased glucose exposure associated with reduced ultrafiltration capacity of the peritoneal membrane. In contrast baseline characteristics such as age, gender and comorbidity or subsequent frequency of peritonitis were not different. These observations have important implications for the longitudinal monitoring, prevention and risk assessment of patients on long-term PD.

A higher risk of EPS has consistently been associated with a longer duration of PD. (200,205) The incidence in this study is in line with this, with a lifetime risk of 8.75% for patients on PD for more than 5 years, a finding in keeping with the incidence reported by the Scottish Renal Registry. (168) One of the strengths of this study is follow up data over a long period of time (19 years) allowing us to examine EPS risk after long periods on PD, and the Kaplan Meier gradient continues to worsen with longer PD exposure, a similar pattern to that published by Kawaguchi et al (206) although in our centre the decline in EPS free survival is shorter.

It has been suggested that transplantation is a particular risk for the development of EPS, (207,208) although this does not seem to be the case for our cohort (12 out of 36 controls vs 0 out of 9 EPS cases had a transplant) nor was it the case in the Pan-Thames EPS study where 14 out of 65 patients had a transplant. (209) The majority of our patients developed the condition after stopping PD (6 out of 9) suggesting that, as has been documented previously, (206,210) cessation of PD may be the main trigger.

The original reports of EPS linked it with an increased rate and/or severity of peritonitis, (205,211–213) although this is not the case in our study. This might be explained by case selection as, to ensure there was uniformity in the diagnosis of EPS, we excluded 2 cases characterised by an early

diagnosis after severe and prolonged peritonitis. However, there have also been other recent case series where the rate of peritonitis was unremarkable such as that from Kawanishi et al (210) where 16 of 50 cases of EPS had no peritonitis, and Summers et al (214) and Hendriks et al (215) where the rate of peritonitis was no different to the entire PD population and control group respectively. These case series suggest infectious peritonitis is no longer such an important feature. Changes in PSTR and UF capacity have been documented with peritonitis, particularly multiple and severe episodes, (41) so there may be a subset of patients in whom severe, non-resolving or recurrent peritonitis is still important in the development of EPS (216) such as the 2 patients we excluded from this analysis.

The PSTR might reflect intraperitoneal inflammation through its association with local IL-6 levels (99) and previous studies have demonstrated a faster PSTR in most patients who develop EPS. (190,215,217) This association with EPS is usually reported in the context of a single measurement at differing time points on dialysis so how this related to the evolution of EPS was unclear until now. Our study confirms previous observations that PSTR tends to increase with time on treatment and shows this was not obviously different between EPS patients and controls, with the exception of the last measurements taken within a year of diagnosis. This is in keeping with previous observations suggesting that the early stages of EPS are inflammatory in nature with evidence of local inflammatory changes in biopsies compared with samples taken from patients with simple peritoneal sclerosis. (165)

Whilst a previous uncontrolled study failed to show an increase in PSTR measured in 4 Standardised Permeability Analyses' prior to the development of peritoneal sclerosis, our controlled study of EPS included measurements from within the final year of PD, the only time point at which there was a significant difference in PSTR. (218) The only previous report of peritoneal protein loss (albumin and IgG) in EPS was in 4 patients with no control group, (217) where 3 had an increase over time and one a decrease. By using a case control design with more cases we have demonstrated that EPS patients actually have a slightly smaller peritoneal protein clearance than time matched controls.

UF failure at the time of PD cessation has been documented as a risk factor for the development of EPS, (215,219) including the same uncontrolled longitudinal study of peritoneal sclerosis mentioned above which demonstrated a decline in UF capacity over 4 years. (218) This EPS study extends these findings in time and by using time matched controls, a clear difference is apparent at least 2 years prior to PD cessation. Our study has also demonstrated for the first time uncoupling of PSTR and UF capacity, with less UF capacity for the same PSTR, in patients who subsequently develop EPS, also a recognised phenomenon in long term PD patients without EPS. (17) A possible explanation for this fall in osmotic conductance is increased fibrosis reducing hydraulic permeability.

Glucose exposure has previously been documented as greater in a group of peritoneal sclerosis patients compared with controls (215) but this study again extends this to a longitudinal description finding a clear separation between cases and controls 4 years prior to stopping PD. Glucose exposure might be a reflection of greater use of APD, but in our study cases and controls had similar rates of APD. Unsurprisingly Icodextrin use was also greater in EPS cases given its known benefits in maintaining PD in patients with ultrafiltration failure, although this was only significant between 1 and 2 years before PD cessation. That glucose exposure is statistically significantly different prior to the divergence in the UF capacity does not necessarily imply that it precedes the deterioration in UF capacity, as there is a small difference in UF capacity at 4 years prior to PD cessation which is almost significantly different ($p=0.07$) and UF capacity in the peritoneal equilibration test is recognised as having a large coefficient of variation, limiting the ability to detect true differences. The pattern of glucose exposure and UF capacity is strikingly similar with the plots in both graphs diverging between 4 and 5 years prior to stopping PD and separating further with time.

The longitudinal evolution of residual renal function has not been studied previously, but this study demonstrated that those patients who go on to develop EPS pass a smaller urinary volume within the first 4 years. This is likely to be another contributory factor, along with declining UF capacity, in explaining the EPS patients' greater and earlier glucose exposure.

The ISPD position paper on PD duration (220) emphasised the desirability of a method of identifying those patients at risk of EPS, not fulfilled by CT scanning, (221) and there is as yet no study on the use of biomarkers in predicting EPS. Patients who lose their residual renal function quickly and have a declining UF capacity, particularly a low UF capacity relative to their PSTR, are the group at highest risk of EPS, and consideration must be given to changing dialysis modality although other important factors must also be considered when discussing this. (220) The glucose exposure may be a more reliable marker than UF capacity as the glucose requirements are likely to be set by the osmotic conductance to glucose of the peritoneal membrane, whilst the measured UF capacity is affected by other issues (e.g. catheter position and drainage) and is thus more variable. This study has partially overcome this issue by using the mean value of twice yearly peritoneal membrane function tests.

From previous studies the PSTR appeared to be an independent predictor of EPS that could be monitored for the purpose of identifying patients at risk. Our data suggests that it would be of less use than glucose exposure or UF capacity as clear separation between EPS cases and controls only appeared in the last year of PD, by which point stopping PD may well be too late, although a rapid increase in PSTR could help identify patients who are already at high risk of EPS at the point of modality switch and therefore require close follow up afterwards.

Conventional glucose-based PD fluid contains glucose degradation products, is acidic, and, particularly for 3.86% bags, possesses an unphysiologically high osmolality, all of which are thought to contribute to membrane damage with an increase in PSTR in conjunction with a worse residual renal function. (42) This study has provided a further rationale to systematically minimise glucose exposure across all PD patients to minimise EPS risk.

There is a growing literature to suggest that ultrafiltration failure in long term PD patients is caused by an increase in fibrosis decreasing osmotic conductance, including evidence from biopsies, (18) from computer modeling (19) and from peritoneal membrane testing. (20) We speculate that this fibrosis explains the changes we have demonstrated in patients who develop EPS, although patients

with these findings have yet to develop EPS suggesting that there is a difference between simple sclerosis and EPS. (165) The PCl data would support this view as, according to the standard 3 pore model, PCl should increase with membrane size and inflammation but the lack of effect we found might be explained by the fiber matrix/3 pore model with fibrosis restricting protein flux across the membrane.

There are some limitations to this study. We could not be certain that all of the patients used as controls would not have gone on to develop EPS as, whilst there was long term follow up data post PD in the majority of the patients in this group, some patients stopped PD as a result of dying of other causes. This would tend to reduce differences between cases and controls but despite this there were still clear differences between the two groups. As with all studies of EPS, it is not helped by the lack of firm diagnostic criteria although the EPS group was carefully limited to those with characteristic CT or operative findings (often both) with typical clinical features and no other apparent cause. It is a single centre study so it is not necessarily widely generalisable, particularly with growing evidence of centre differences, (222) although our data was consistent with the existing literature. There is also the possibility that cases were not diagnosed, although this should only significantly affect the incidence and this was similar to that found in the most comparable population studied. (168)

In summary, we report in detail the longitudinal changes in peritoneal membrane characteristics in the majority of patients who subsequently develop EPS (not associated with severe or prolonged peritonitis), demonstrating that loss of UF capacity is the predominant early change noted associated with lower initial residual renal function and higher glucose and Icodextrin exposure. Patients with these features are at high risk of EPS and consideration should be given to stopping PD, while these features and a rapid PSTR at the time of PD cessation should prompt close monitoring for EPS post-PD.

8 Competing Risks of Encapsulating Peritoneal Sclerosis

8.1 Summary

8.1.1 Introduction

Encapsulating peritoneal sclerosis (EPS) is an uncommon complication of peritoneal dialysis (PD), where the risk increases significantly with increasing time on therapy. As risk factors for the competing event of death seemed likely to decrease the risk of developing EPS, we performed a competing risks analysis prior to developing a prognostic model from this..

8.1.2 Methods and Materials

We combined 3 large datasets (AnzData, Global Fluid Study, Scottish Renal Registry (SRR)) with complete data on EPS occurrence and the denominator population. All incident patients aged ≥15 years were included and a competing risks survival analysis used with outcomes of censored, EPS (prior to death) or death and robust standard errors. Comorbidity data was classified by either primary renal diagnosis (low comorbidity = glomerulonephritis, polycystic kidney disease, chronic pyelonephritis, high comorbidity = other) and diabetic status (all 3 datasets) or by Stoke comorbidity score (AnzData and Global).

8.1.3 Results

There were 112 cases of EPS out of 17,912 patients. The cumulative incidence at 10 years varied from 0.04 in AnzData, to 0.25 in SRR. Competing risks models showed age (SHR 0.79 per decade, 95% CI 0.5-0.83) and high comorbidity renal disease (SHR 0.54, 95% CI 0.41-0.73) decreased the risk of EPS which Cox models failed to demonstrate. The SRR had a SHR of 5.62 (95% CI 5.28-6.21) relative to AnzData but this was not through a decreased mortality (HR for mortality in SRR vs AnzData in adjusted Cox model 1.14, 95% CI 1.05-1.42) or through longer periods of PD (median months on PD, SRR 22.6, AnzData 21.1, p=0.2). The Global dataset had an intermediate risk (SHR

relative to AnzData 2.11, 95% CI 1.78-2.49) but the numbers were small so no further analysis was performed on this.

8.1.4 Discussion

For patients commencing PD, factors that increase the risk of death decrease the risk of developing EPS. Competing risks regression is an appropriate model for analysis of dialysis outcomes. The Scottish Renal Registry has a significantly higher rate of EPS than found in AnzData, possibly due to ascertainment bias or genetic factors.

8.2 Introduction

EPS is a severe complication of PD, with a significant impact on morbidity and mortality. It is most strongly associated with a prolonged period of time on PD (205,223) so tends to affect younger and 'fitter' patients who survive on PD for a sufficient length of time to be at significant risk of EPS. There is a concern that a large proportion of patients, to avoid the perceived risk of EPS, will be transferred from PD, their preferred dialysis modality, to HD, when only a very small proportion of those patients would have gone on to develop EPS. A prognostic model informing physicians of a patient's risks of death and EPS would help discussions of switching to HD with patients likely to develop EPS, and of staying on PD for patients likely to die (whether or not they switch) rather than get EPS.

Commonly used survival analyses have relied on the assumptions behind the Cox model and the Kaplan-Meier estimator, where the event of interest is treated as inevitable and any cases who have not experienced the event of interest at the time of the study ending are treated as censored. This means that censored cases are removed from the risk set, which falsely increases the predicted risk of the event of interest at any one time. In the case of EPS, patients who have died are removed from the risk set so the estimated risk of EPS is then directly related to the relative numbers of EPS cases at any time point compared to patients still on PD who have not been censored, rather than

the proportion of cases compared to a larger population. All previous studies of EPS have used these survival analyses so will tend to overestimate the true risk of EPS.

Competing risks analysis has been developed as a partial solution to these problems, where there is more than one possible outcome and these outcomes are mutually exclusive. (224) By keeping patients with competing outcomes in the risk set, the estimated probability of an event is more realistic, although the estimates are usually under the 'true' probability. It can also provide the overall effect on an outcome of interest of a variable, where it may change the rate of both the event of interest and the competing event. For EPS, death is a strong competing risk. We sought to perform a competing risks analysis, with the intention of subsequently creating a prognostic model.

8.3 Methods and Materials

8.3.1 Study Design

This chapter describes a competing risks regression analysis for the events of death prior to EPS and EPS prior to death, using data from AnzData and the SRR with variables that could be included in a prognostic model.

Only variables common to both datasets were included, these being age, diabetes gender and primary renal disease. Primary renal disease is known to predict mortality so adult polycystic kidney disease, glomerulonephritis and chronic pyelonephritis were categorised into a low mortality risk group and all other causes were considered high risk. The standard ethical and governance processes for each data source were followed.

8.3.2 Data sources

The AnzData registry includes all patients who have ever had RRT in Australia or New Zealand and they supplied data on all patients commencing PD. Cases of EPS were identifiable from 3 sources in

the routinely returned registry data: new comorbid conditions, causes of death and reasons for stopping PD, the latter 2 of which contain EPS specific codes. Primary renal disease, age, gender, comorbidity data and dates of PD start and cessation were all available. Only patients commencing RRT from 1/1/1990 were included in this study.

The Scottish Renal Registry includes all patients on RRT in Scotland from 1991. EPS cases from a cohort of patients commencing PD between 1/1/2000 and 31/12/2007 were identified for a previous study (220). 35 cases were identified from follow up of this cohort until 30/6/2011. Registry data included age, gender, primary renal disease and dates of PD start and cessation.

The GFS was an observational cohort study of incident and prevalent patients with 11 identified cases of EPS from 5 UK centres with complete PD patient data but this was considered too few cases to justify including a further dataset.

8.3.3 Competing risks analysis

For the initial analysis, we included all patients at the point of starting PD. Competing risks models according to Fine and Gray were developed with covariates included as above as well as a dataset identifier. The primary outcome was EPS occurring prior to death, with death prior to EPS the competing event. The covariates used were primary renal disease as a binary variable, age, dataset (AnzData/SRR), gender and the presence of diabetes.

To compare the effects, standard Cox models for EPS and for death were also run. StataIC 12.1 (College Station, Texas) was used for all analyses. Proportional hazards were checked with Schoenfeld residual plots and DFBetas were calculated to check for influential points (these checks were performed by Lucy Riley).

8.4 Results

The study populations are described in Table 8-1. The populations were broadly similar although the AnzData patients were slightly older, with a slightly shorter mean follow up period. The rate of diabetes was significantly higher which may have contributed to the small difference in rates of 'high comorbidity' primary renal disease. The rates of EPS, not corrected for duration of PD or follow up period, were significantly different: AnzData 0.47% (77/16,274) and SRR 2.7% (34/1,237).

Table 8-1: Characteristics of Study Populations

Variable	SRR	AnzData	p value
Number of Patients	1,237	16,274	
EPS Cases	34	77	
Age	55.1	58.8	<0.001
Male Gender	55.0%	54.8%	0.98
Mean follow up period	64.5	60.7	<0.001
Median (IQR) Duration of PD	22.6 (9.2-37.7)	21.1 (9.5-38.6)	0.2
Median (IQR) Days on RRT prior to PD	0 (0-27)	0 (0-41)	<0.001
High comorbidity primary renal disease	59.9%	62.8%	0.04
Diabetes	26.6%	40.3%	<0.001

Table 8-2: Competing Risks Model for Predictors of EPS

Variable	Sub-Distribution Hazard Ratio (95% CI)	p value
Age	0.976 (0.967-0.986)	<0.001
Male Gender (vs female)	0.95 (0.65-1.38)	0.8
High Risk Primary Renal Disease (vs low risk)	0.52 (0.32-0.84)	0.008
Diabetes (vs no diabetes)	0.84 (0.48-1.47)	0.5
Scottish Renal Registry (vs AnzData)	5.63 (3.78-8.38)	<0.001

The results from the competing risks analysis of EPS are shown in Table 8-2. As expected, gender was not significant, whilst increased age and high risk primary renal disease decreased the risk of EPS. Diabetes had no significant effect on risk of EPS but the risk of EPS between datasets was greatly

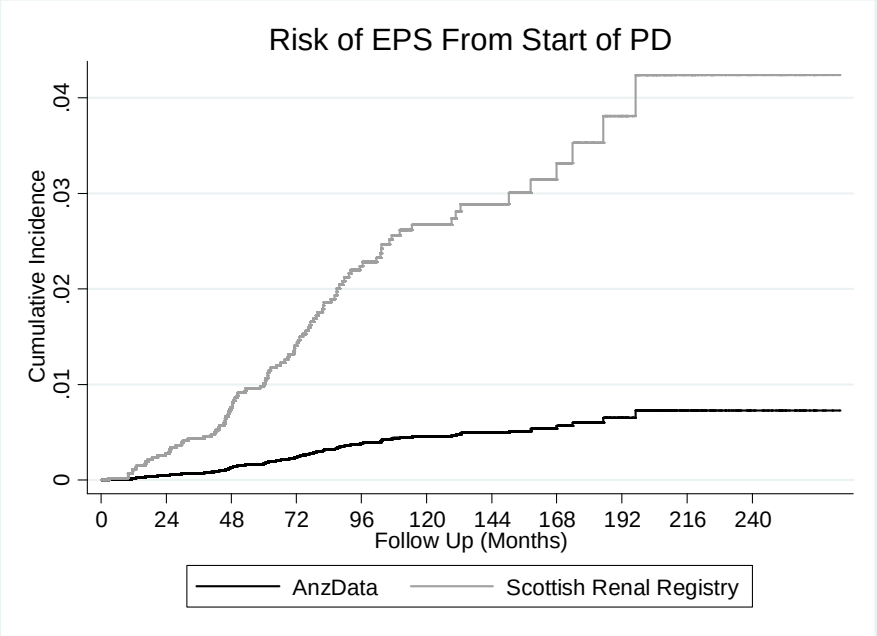
different, as demonstrated in Figure 8-1. A sensitivity analysis to check for a period effect was performed, restricting the AnzData patients to those starting PD from 1/1/2000 onwards. The same pattern was observed. No interactions between variables were observed.

The Cox models with cause-specific hazards are shown in Table 8-3. For EPS, the effect of age disappears and that of high risk primary renal disease becomes statistically insignificant, but the dataset effect remains highly significant. The model for death showed that high risk primary renal disease, diabetes, increased age, female gender and being in the Scottish dataset were associated with increased risk of mortality.

Table 8-3: Cox Models for Predictors of EPS and Death

	EPS		Death	
Variable	**Hazard Ratio (95% CI)**	**p value**	**Hazard Ratio (95% CI)**	**p value**
Age (per year)	1.00 (0.99-1.01)	0.98	1.046 (1.044-1.048)	<0.001
Male Gender (vs female)	0.98 (0.67-1.43)	0.92	0.94 (0.91-0.98)	0.004
Scottish Dataset (vs AnzData)	6.19 (4.06-9.42)	<0.001	1.14 (1.05-1.24)	0.001
Diabetes (vs no diabetes)	1.10 (0.65-1.88)	0.71	1.45 (1.39-1.52)	<0.001
High risk primary renal disease (vs low risk)	0.68 (0.43-1.07)	0.092	1.55 (1.47-1.63)	<0.001

Figure 8-1: Cumulative Incidence of EPS By Dataset



8.5 Discussion

This analysis has proven the basic premise behind the intended prognostic model, that the risk of EPS can be predicted by factors associated with increased mortality. We have also demonstrated for the first time a significant difference in apparent risk of EPS by geographic region.

That a competing risks analysis is appropriate for EPS in certain situations is demonstrated by the differences between the results of the competing risks and Cox analyses. Physicians and patients are interested in 'real world' risk factors, which include the effect of variables acting via alternative outcomes as provided by the competing risks analysis here. Cox models describe variables acting directly upon the outcome of interest, which may be of more interest if seeking pathophysiological insights.

When comparing our results to existing literature, there is a significant problem in that we have not included the strongest known predictor of EPS, duration of PD, in our model. This is because inclusion of time varying covariates in a competing risks model requires knowledge of the covariate values after occurrence of the competing event that lead up to the eventual primary outcome. (225) In our case, this would require knowledge of what duration of time on PD leads to EPS after death has occurred, which is clearly impossible.

The effect of age is compatible with previous findings as evidenced by relatively young mean ages (42 and 43.4 years old at diagnosis) in published case series (191,214) compared with typical ages for commencing dialysis, as well as a younger age in a case-control study from the AnzData registry. (226) It is also consistent with the multivariable analysis conducted with SRR data where age was not significant as logistic rather than competing risks regression was used. (168) Measures of comorbidity, including surrogates like the 'high risk primary renal diagnosis' in this study, have not been assessed in previous reports.

The lack of effect of diabetes is not surprising. The majority of diabetics in renal registries have diabetic nephropathy listed as their primary renal disease, (227) and would therefore be coded as 'high risk primary renal disease' in this study. An independent effect of diabetes on EPS would be difficult to ascertain, given that it does not directly increase the risk of EPS. The sub-distribution hazard ratio for diabetes was below one but even using the largest study of EPS to date, the confidence intervals around diabetes remain wide.

We have demonstrated the effect of time on the incidence of EPS (Figure 8-1), and the effect appears to differ from previously published reports in that the slope is approximately linear compared to slopes that increase with time. (168,205,206) This could be due to the use of the competing risks cumulative incidence function, rather than Kaplan-Meier estimators, or it could be due to the use of time, rather than time on PD. The relative roles of these explanations will require formal modelling of time on PD whilst taking account of the competing risk of death.

The marked difference in risk between AnzData and SRR datasets is pronounced, which requires further investigation as these 2 datasets have led to some of the main publications that inform our current estimates of the magnitude of the problem of EPS. The difference could have several explanations. Firstly, a genetic difference cannot be excluded by this study. Secondly, a difference in time on PD is possible although there was no difference in median time on PD between datasets. A more subtle effect such as better recognition of patients at risk of EPS in the AnzData region cannot be excluded and would require formal modelling of the effect of time on PD (e.g. in a multistate model). This explanation seems unlikely as there was limited recognition of EPS outside of Japan in the 1990's.

Thirdly, if patients in the AnzData region were significantly more likely to die, their risk of EPS would be lower but the Cox model demonstrates that patients from Scotland have a significantly higher risk of death. Finally, ascertainment bias may explain the difference completely. AnzData rely on routinely returned data with EPS coded as either the reason for stopping PD, the cause of death or

as a new comorbid condition whereas the SRR data identified EPS cases by specifically contacting each of the 10 units to ask for all cases of possible EPS. Aside from potential problems with the accuracy of routine registry data, EPS may well have milder forms that lead to diagnostic uncertainty and under-reporting in units less familiar with an unusual condition. (228)

Limitations of this study include those discussed above to explain the differences between datasets, as well as the limited number of variables shared between datasets. The number of EPS cases is still relatively small, despite this study including the largest number of EPS cases in published studies to date, which will decrease the power to detect true associations. We have so far not included the effect of time on PD in the statistical model. Due to some of these limitations we have not yet established the 'true' baseline risk of EPS, which will require validation with other data sources.

In summary, we have established the validity behind the concept of a prognostic model of EPS risk based on a competing risks methodology and uncovered important differences in apparent risk between 2 of the datasets used to inform current estimates of EPS risk.

9 Systemic effects of peritoneal dialysis

9.1 Summary

9.1.1 Background

Glucose control is a significant predictor of mortality in diabetic peritoneal dialysis (PD) patients. During PD, the local toxic effects of intra-peritoneal glucose are well recognized, but despite large amounts of glucose being absorbed, the systemic effects of this in non-diabetic patients are not clear.

9.1.2 Methods and Materials

We analysed the Global Fluid Study, a prospective, observational cohort study initiated in 2002. A subset of 10 centres from 3 countries with high data quality were selected (368 incident and 272 prevalent non-diabetic patients), with multilevel, multivariable analysis of the reciprocal of random blood glucose levels, and survival analysis by stratified-by-centre Cox regression model.

9.1.3 Results

The median follow up was 5.6 and 6.4 years respectively in incident and prevalent patients. Levels suggested undiagnosed diabetes in 3.7% and 5.4% of incident and prevalent patients respectively. On multivariable analysis glucose levels decreased with higher plasma sodium (β=0.002, 95%CI 0.0005, 0.003) in incident patients, increased with age in incident (β=-0.007, 95%CI -0.01, -0.004, p<0.001) and prevalent (β=-0.004, 95%CI -0.008, -0.002, p=0.04) groups and increased with total 24 hour dialysate glucose load (β=-0.0003, 95%CI -0.0005, -0.00001, p<0.001) in prevalent patients. For prevalent patients on Icodextrin a U-shaped association between random blood glucose and dialysate glucose was significant. Glucose levels predicted death in unadjusted analyses of both incident and prevalent groups but in an adjusted survival analysis they did not (for random blood glucose 6-10 compared with <6, Incident group HR 0.92, 95%CI 0.58, 1.46, Prevalent group HR 1.42,

95%CI 0.86, 2.34).

9.1.4 Discussion

In prevalent patients, random blood glucose levels are higher with increased total dialysate glucose load, and levels compatible with diabetes are under-recognised. Random blood glucose levels predict mortality in unadjusted analyses, but this association has not been proven in adjusted analyses.

9.2 Introduction

There is a large amount of laboratory and clinical evidence of glucose-based peritoneal dialysate causing significant damage to the peritoneal membrane (56,229) but there have been far fewer studies documenting the systemic consequences of glucose-based dialysate. Significant glucose absorption from the peritoneum occurs during PD, such that glucose induced hyperosmolarity prevented the use of higher dialysate glucose concentrations. (230)

Insulin resistance, along with hypertriglyceridaemia, high HDL/low LDL-cholesterol, hypertension and abdominal obesity, are defined as metabolic syndrome (MetS), (231,232) a condition thought to be related to sustained high sugar intake in the general population (233) and which predicts cardiovascular mortality. (232) Impaired fasting glucose predicts mortality, although not as well as impaired glucose tolerance, (234) and impaired fasting glucose increases during PD by up to 49.8%, along with other features of MetS.(185) These changes are all associated with glucose exposure, apart from impaired fasting glucose but this negative association was of glucose levels with historical glucose exposure, rather than a contemporaneous measure of glucose exposure. High glucose levels in PD patients are associated with mortality on univariable analysis (235) so whether a reduction in dialysate glucose exposure can mitigate the increase in hyperglycaemia is an important clinical question.

We hypothesised that a contemporaneous measure of dialysate glucose loading would be associated with systemic glucose levels, and that impaired glucose homeostasis would independently predict mortality in non-diabetic patients. We used the GLOBAL Fluid Study to address these questions.

9.3 Methods and Materials

Study design

The study has been described in detail elsewhere but in brief, the Global Fluid Study is an international, multicentre, prospective cohort study of incident and prevalent patients commenced in 2002. Eligible patients were any PD patients over the age of 18 providing informed consent. Incident patients were defined as first data collection time point within the first 90 days of PD. Follow up was censored in December 2011. Ten centres were selected based on the highest quality existing data then iteratively checked to optimise final data completeness, and a cross-section of all non-diabetic patients from these units was used for this analysis at the point of study entry. Despite this process, one centre had significantly worse data quality in the final analysis, so sensitivity analyses excluding this centre were pre-specified.

Data collection

All clinical data was recorded on a custom built database (PDDB). Demography was recorded and comorbidity was assessed with the validated Stoke comorbidity index. Routine blood tests, including albumin and random blood glucose, were performed locally and, if necessary, converted into the same units. The timing of the plasma samples was not specified during the study so most were taken during peritoneal equilibration testing, but a few were taken during adequacy sampling, and none of them were specifically fasted samples. The samples of dialysate and plasma taken at the first assessment within the study were assayed for IL-6 by electrochemiluminescence.

PD related measurements included residual renal function, dialysis regime and dose, and peritoneal membrane function using the peritoneal equilibration test (solute transport rate: dialysate to plasma creatinine ratio (PSTR) and net UF capacity at 4 hours with 2.27% or 3.86% glucose). The Daily Dialysate Glucose (DDG) exposure was calculated as total grammes of unhydrated glucose within the

24 hour dialysate regime as recorded on the day of assessment (e.g. 2 litres of 1.36% glucose based dialysate = 2 x 13.6 grammes = 27.2 grammes)

Statistical analysis

Comparisons between glucose categories were made with one-way ANOVA, Kruskal-Wallis or chi-squared tests depending on the variable.

Missing data, ranging from 0 to 4.8% for different variables, were considered missing at random and complete case analysis was used. Pre-specified sensitivity analyses excluding one centre with the highest level of missing data were performed.

Multivariable multilevel, random intercept models, which account for centre effects, were used to explore determinants of random blood glucose. To achieve normally distributed level 1 residuals, the reciprocal of glucose was used as the dependent variable. Significance testing was by the Wald test. The Iterative Generalised Least Squares method was used for coefficient estimation.

Log-rank tests were used for univariable, and Cox models stratified by centre with robust standard errors were used for multivariable, survival analysis. To allow for non-linearity glucose levels were categorised into <6 mmol/l, 6-10mmol/l and >10 mmol/l, the levels chosen to aid biological interpretability whilst maintaining group size. A secondary analysis with glucose included as a continuous variable in the same adjusted Cox model was also performed.

MLWin 2.26 was used via runmlwin for multilevel regression and StataIC 12 (StataCorp LP, College Station, TX) for the other calculations.

9.4 Results

9.4.1 Patient Details

Demographic and clinical data are shown in Table 9-1. There were 576 incident patients, with glucose available in 548, of whom 327 were non-diabetic with 116 deaths during a median follow up of 5.93 years. There were 384 prevalent patients, with glucose available in 360, of whom 242 were non-diabetic with 96 deaths during a median follow up of 7.29 years. In incident patients, glucose levels were ≥6.2 mmol/l in 29.8%, 7.0-11.1mmol/l in 15.3% and >11.1mmol/l in 3.7%, and in prevalent patients they were ≥6.2 mmol/l in 32.6%, 7.0-11.1mmol/l in 14.0% and >11.1mmol/l in 5.4%. Outcome data was unavailable for 8 prevalent patients from one centre and 16 incident patients (15 from the same centre), but sensitivity analyses excluding this centre entirely made no difference.

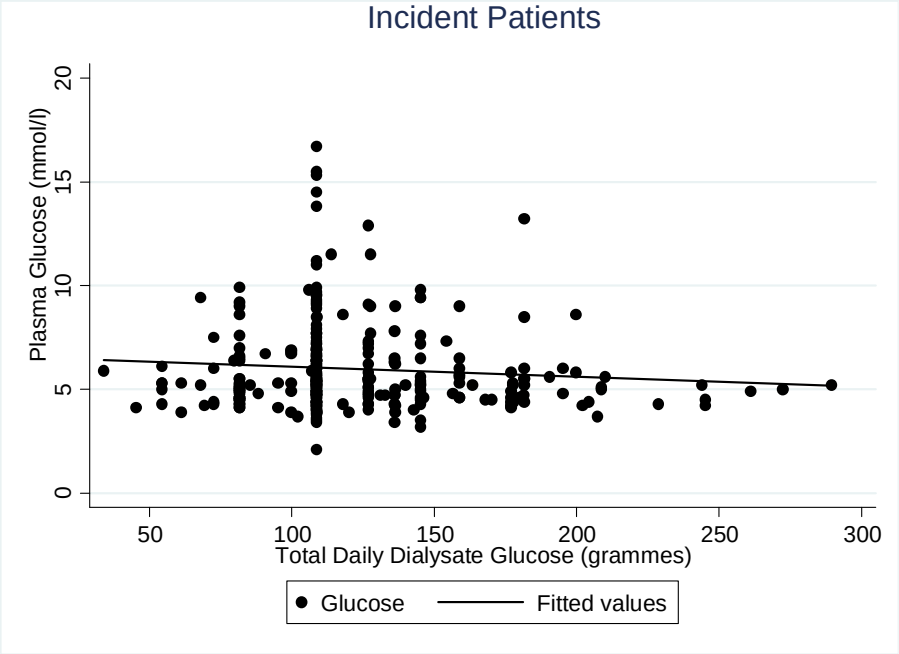
9.4.2 Factors affecting systemic glucose levels

Using multilevel multivariable models, in incident patients DDG load did not predict glucose levels on unadjusted or adjusted analysis (Figure 9-1 and Table 9-2). On both unadjusted (β=-0.003, 95% CI (-0.005,-0.001), p=0.001) and on adjusted analysis (Figure 9-1 and Table 9-2), the DDG load predicted random blood glucose levels in prevalent patients. Sensitivity analyses excluding one centre with higher levels of missing data slightly altered the results in prevalent patients, by strengthening the association with DDG and weakening it with albumin. We also tested for 3 specific interactions - between DDG and peritoneal solute transport rate, DDG and the type of PD (automated PD versus continuous ambulatory PD), and DDG and Icodextrin usage. The first 2 had no significant effect, but a linear interaction with Icodextrin usage did and this improved with the addition of a quadratic term. Predicted values from the regression model on or off Icodextrin are

Table 9-1: Patient Details

	Incident					Prevalent				
	All	**Glucose <6**	**Glucose 6-10**	**Glucose >10**	**p value**	**All**	**Glucose <6**	**Glucose 6-10**	**Glucose >10**	**p value**
Number	327	219	95	13		242	151	74	17	
Age (years)	55.1 (17.0)	52.7 (17.4)	59.9 (15.6)	59.7 (12.2)	**0.002**	53.3 (15.8)	52.0 (16.2)	55.3 (15.5)	56.5 (12.8)	0.1
Female Gender	34.3%	36.2%	40.6%	46.2%	0.4	47.9%	49.7%	47.3%	35.9%	0.5
Korean	37.4%	33.0%	42.6%	76.9%	**0.002**	34.7%	29.1%	41.9%	52.9%	0.1
Duration of PD (median, days)	38	36 (22-52)	42 (29.5-51.5)	36 (28.5-51)	0.1	369 (177-819)	377 (193-818)	358 (162-716)	371 (167-605)	0.7
BMI (kg/height^2)	24.8 (4.8)	24.6 (4.9)	25.3 (4.7)	23.9 (3.8)	0.4	24.6 (4.4)	24.4 (4.0)	25.3 (4.9)	22.9 (3.9)	0.1
Blood pressure (mmHg)	135/81 (20/12)	135/82 (20/12)	136/79 (22/13)	137/80 (24/14)	0.6/0.3	134/82 (21/13)	135/82 (22/14)	132/81 (17/10)	136/83 (23/13)	0.6/0.7
4 hour PSTR (D/P Cr)	0.69 (0.12)	0.69 (0.13)	0.69 (0.10)	0.71 (0.13)	0.9	0.70 (0.11)	0.71 (0.12)	0.70 (0.11)	0.73 (0.13)	0.5
Albumin (g/l)	36.1 (5.0)	36.5 (5.0)	35.5 (4.7)	34.2 (5.7)	0.1	36.1 (4.7)	36.7 (4.4)	35.1 (4.7)	33.9 (5.6)	**0.005**
Urine volume (median, litres)	0.9 (0.48-1.52)	0.99 (0.50-1.70)	0.85 (0.49-1.21)	0.6 (0.20-1.24)	0.2	0.52 (0.15-1.13)	0.57 (0.20-1.21)	0.55 (0.14-1.15)	0.3 (0.02-0.93)	0.3
Comorbidity (Low/Intermediate/High)	56.8, 31.0, 12.3%	57.3, 39.9, 2.8%	56.4, 41.5, 2.1%	53.8, 38.5, 7.7%	0.95	62.8, 34.3, 2.9%	64.9, 32.5, 2.6%	60.8, 35.1, 4.1%	52.9, 47.1, 0%	0.8
Plasma IL-6 (median, pg/ml)	1.27 (0.55-2.75)	1.2 (0.50-2.55)	1.73 (0.66-3.13)	1.75 (0.74-2.74)	0.1	1.1 (0.58-2.00)	0.94 (0.54-1.62)	1.51 (0.82-2.71)	0.95 (0.71-1.51)	**0.004**
Total Daily Dialysate Glucose (grammes/day)	120.8 (36.8)	123.8 (41.2)	114.5 (25.9)	117.6 (20.4)	0.2	132.0 (45.8)	128.7 (38.9)	136.2 (50.0)	142.7 (74.4)	0.3
Total dialysate volume (litres)	7.98 (1.28)	7.97 (1.47)	8.02 (0.86)	7.93 (0.25)	0.8	8.35 (1.95)	8.37 (2.06)	8.32 (1.83)	8.29 (1.57)	0.98
Biocompatible solution usage	23.8%	25.3%	18.1%	38.5%	0.2	16.2%	16.7%	13.5%	23.5%	0.6
Icodextrin solution usage	14.8%	15.2%	16.0%	0%	0.3	23.8%	25.5%	23.0%	11.8%	0.5
Use of APD	6.5%	8.4%	3.2%	0%	0.09	15.8%	16.7%	15.1%	11.8%	0.9

Figure 9-1: Scatterplot of Plasma Glucose vs. Total Daily Dialysate Glucose in Prevalent Patients



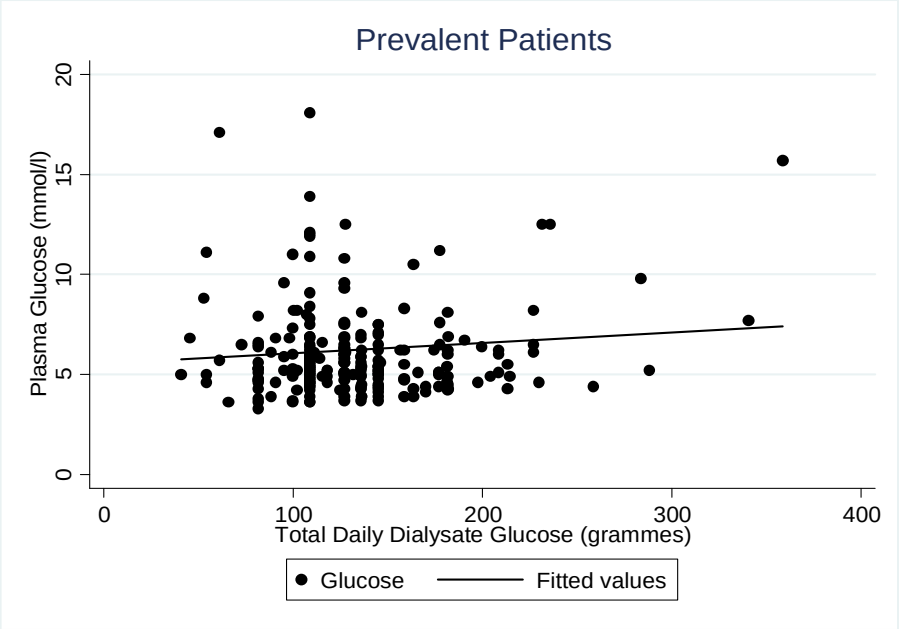
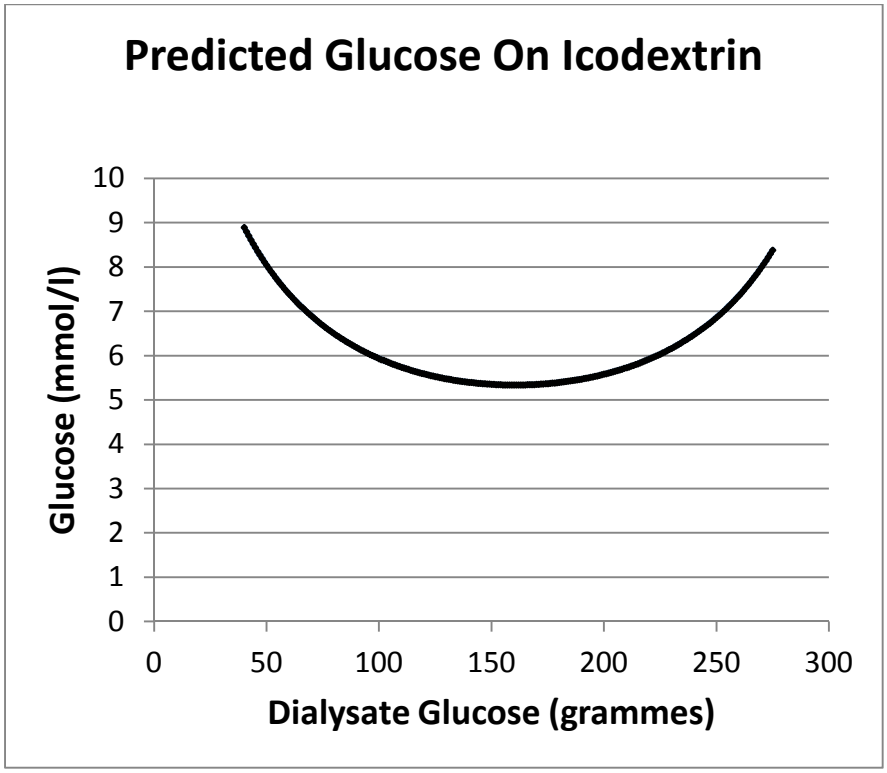
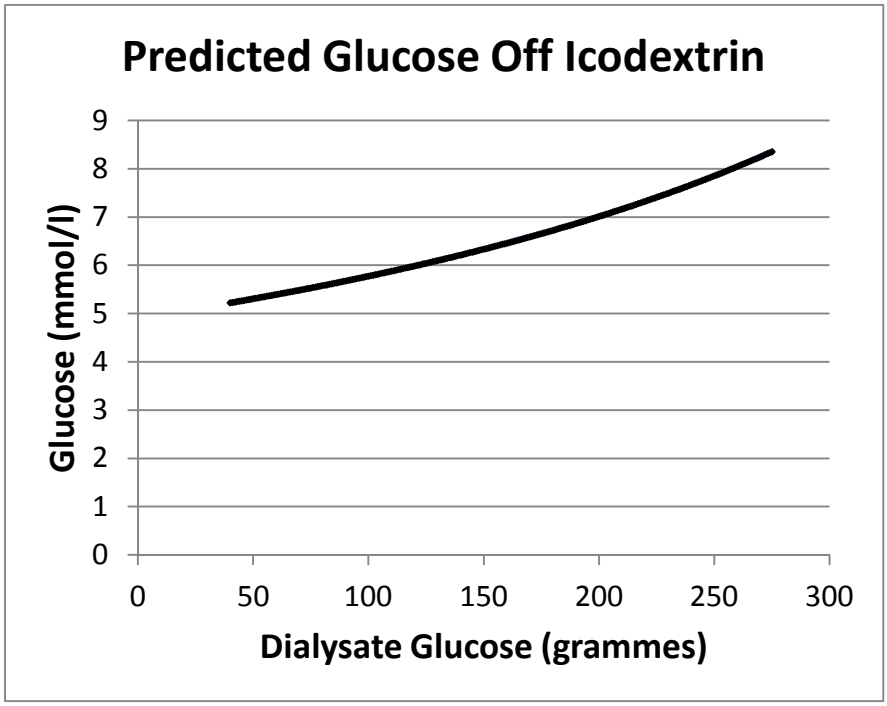


Table 9-2: Predictors of the Reciprocal of Random Blood Glucose Levels

	Incident		Prevalent	
	Coefficient (95% CI)	**p value**	**Coefficient (95% CI)**	**p value**
Daily Dialysate Glucose	**0.0001** (-0.00002, 0.0003)	0.09	**-0.0003** (-0.0005, -0.00001)	**<0.001**
Korean	**-0.03** (-0.05, -0.001)	**0.04**	-0.01 (-0.05, 0.03)	0.6
BMI	-0.0008 (-0.0020, 0.0003)	0.1	-0.0006 (-0.002, 0.0007)	0.4
Age (per year)	**-0.0007** (-0.0010, -0.0004)	**<0.001**	**-0.0004** (-0.0008, 0.0002)	**0.04**
Systolic BP (per 10)	-0.001 (-0.004, 0.001)	0.3	0.002 (-0.006, 0.005)	0.1
Peritoneal Solute Transport Rate	0.04 (-0.01, 0.09)	0.2	-0.03 (-0.08, 0.03)	0.4
Duration of PD	-0.006 (-0.14, 0.002)	0.2	-0.0002 (-0.004, 0.003)	0.9
Albumin	0.0001 (-0.001, 0.001)	0.8	0.001 (-0.0003, 0.002)	0.1
Plasma Sodium	**0.002** (0.0006, 0.003)	**0.005**	0.002 (-0.0002, 0.003)	0.08
Plasma IL-6 (per log_{10} order)	-0.003 (-0.02, 0.01)	0.8	-0.01 (-0.04, 0.01)	0.4
Urine Volume	0.002 (-0.006, 0.009)	0.6	-0.001 (-0.01, 0.01)	0.8
Comorbidity	-0.00006 (-0.007, 0.007)	0.99	0.004 (-0.003, 0.01)	0.3
Icodextrin	0.008 (-0.007, 0.023)	0.3	**-0.15** (-0.24, -0.06)	**0.01**
Icodextrin*Dialysate Glucose **Icodextrin*Dialysate Glucose^2**			**0.002** (0.0007, 0.003) **-0.000005** (-0.000009, -0.000001)	**0.002** **0.01**

Figure 9-2: Icodextrin Effect on Plasma Glucose Interacting with Dialysate Glucose





9.4.3 Effect of glucose on mortality

Kaplan-Meier plots of survival in non-diabetic patients by random blood glucose categories are shown in Figure 9-3 and Figure 9-4, and they both demonstrated a significant difference on unadjusted analysis. Table 9-3 shows the results of adjusted Cox models for the same groups, whereby the effect of glucose on mortality is completely removed by adjustment in incident patients. In prevalent patients there is still a trend towards higher mortality but it is not statistically significant.

In a secondary analysis, glucose was included as a continuous variable in the same adjusted model and it was not statistically significant for incident patients (HR 1.0002 for 1mmol/l increase, 95% CI (0.97, 1.03), p=0.993) or for prevalent patients (HR = 1.035 for 1mmol/l increase, 95% CI (0.96, 1.11), p=0.4).

Figure 9-3: Survival by random blood glucose in Incident non-diabetic PD patients

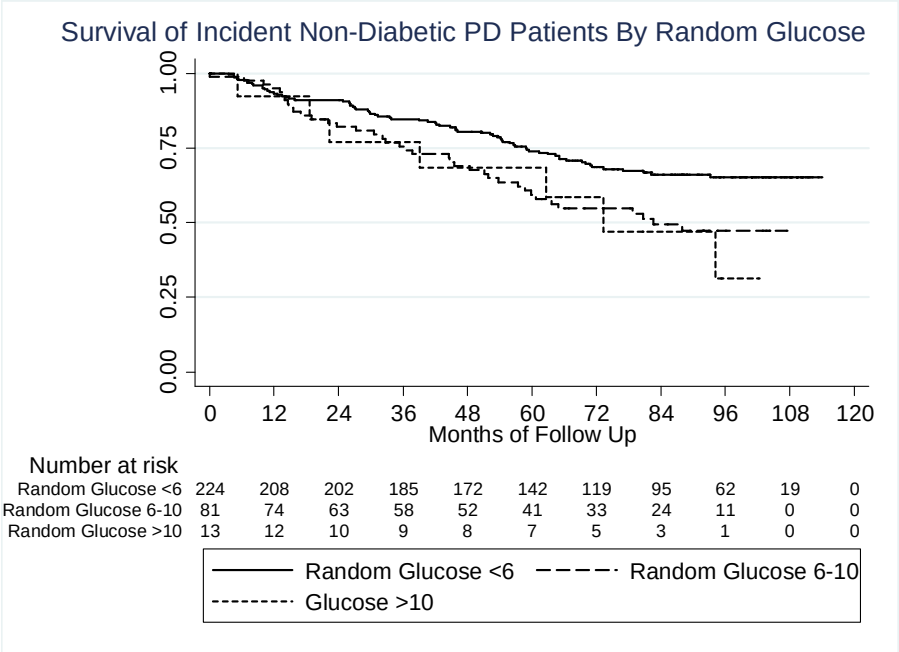


Figure 9-4: Survival by Random Blood Glucose in Prevalent Non-Diabetic PD Patients

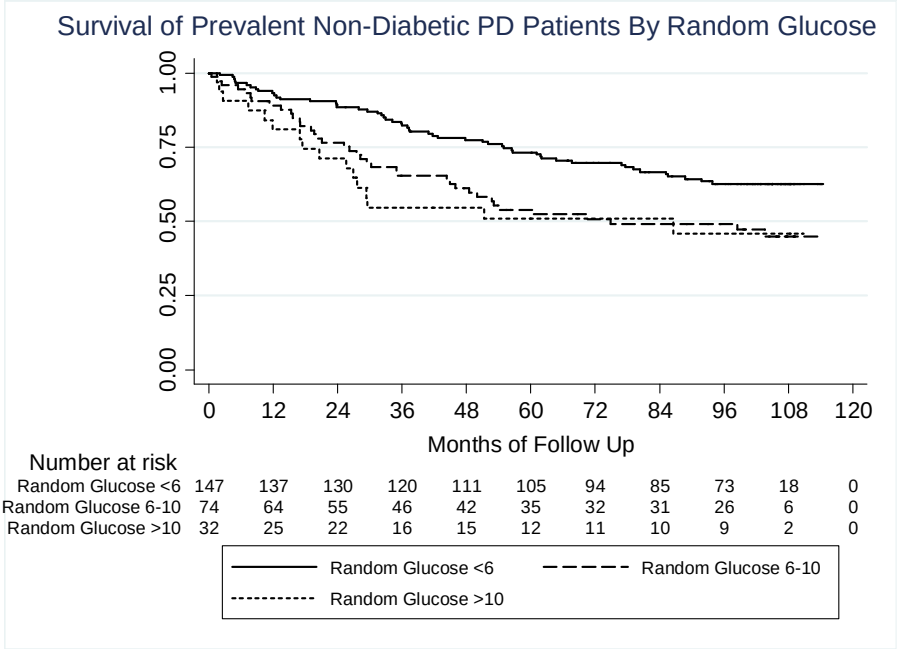


Table 9-3: Predictors of Mortality

	Incident Patients		Prevalent Patients	
	Hazard Ratio (95% CI)	**p value**	**Hazard Ratio (95% CI)**	**p value**
Age (per decade)	**1.92** (1.51-2.41) **	<0.001	**1.78** (1.48-2.14)**	<0.001
Plasma IL-6 (per log order)	**3.07** (1.70-5.54)**	<0.001	**3.08** (1.22-7.79)*	0.02
Albumin	**0.96** (0.92-0.999)*	0.049	0.97 (0.93-1.02)	0.3
PSTR (per 0.1 increased D/P Cr)	1.05 (0.91-1.21)	0.5	**1.24** (1.02-1.50)*	0.03
Duration of PD (per month)	1.14 (0.70-1.85)	0.6	**1.013** (1.003-1.023)*	0.01
RRF (per litre of urine volume)	0.80 (0.60-1.08)	0.1	0.65 (0.41-1.03)	0.06
Comorbidity	**1.48** (1.18-1.86)**	0.001	**1.39** (1.15-1.69)**	0.001
Glucose (ref ≤6)				
Glucose >6, <10	0.95 (0.59-1.51)	0.8	1.45 (0.90-2.36)	0.13
Glucose ≥10	1.15 (0.58-2.29)	0.7	1.32 (0.54-3.27)	0.5

9.5 Discussion

This is the first study to demonstrate an effect of dialysate prescription on systemic glucose metabolism in non-diabetic patients, with higher random blood glucose levels correlating with increasing dialysate glucose exposure. We have also replicated the finding of unadjusted higher mortality rates with higher plasma glucose levels in a more widely generalisable population than shown previously, (235) although this association was not statistically significant in a more fully adjusted analysis than has been possible previously.

The American Diabetic Association (ADA) defines impaired glucose metabolism as either impaired fasting glucose or impaired glucose tolerance (236). This makes the interpretation of all studies on this subject in PD patients limited by the glucose absorption which occurs during PD because, as the level of absorption is not usually known at the point of sample acquisition, it cannot be truly considered fasting unless PD is withheld, and a glucose tolerance test requires a defined dose supplied enterally. However the large studies necessary to detect subtle effects require straightforward tests. Our study used a pragmatic solution of completely random blood glucose levels and other studies opted for orally but not peritoneally fasted glucose levels although the diagnostic criteria for impaired fasting glucose or impaired glucose tolerance in the general population cannot be assumed to hold for either of these options in PD patients.

Previous studies of abnormal glucose metabolism have shown that transplant wait-listed PD patients in the US had a similar but lower incidence of diabetes than haemodialysis patients (237) however a study of 195 non-diabetic Chinese patients found an increase from 12.8% to 62.6% in impaired fasting glucose (>6.2mmol/l) during 34 months of PD (185) and a study of 252 non-diabetic Chinese patients found new onset impaired fasting glucose (7.0-11.1mmol/l) in 19.0% after 1 month of PD**.** (235) Our study had a much lower rate of glucose levels >6.2mmol/l compared to 62.6%. (185) This was despite our patients not orally fasting although our study sampled the patients after only 12 months of PD. Our results in incident patients are similar to, but lower than, those from Szeto et al.

(235) These rates in conjunction imply that oral fasting does not have a large impact on the glucose levels in PD patients.

Both our study and that of Szeto et al suggest that undiagnosed diabetes is an issue in PD patients. The ADA diagnostic criteria (236) use casual glucose levels >11.1mmol/l with suggestive symptoms to diagnose diabetes and neither of these 2 studies had details of symptoms but Szeto found 8.3% in an incident group (235) and we found 3.7% and 5.4% in incident and prevalent groups respectively to have levels compatible with diabetes.

In line with results from Szeto, (235) age was predictive of glucose levels in patients after 1 month of PD but using a multivariable model we also found plasma sodium to have a strong negative association with glucose. An association between hyponatraemia and hyperglycaemia is well recognised in other patient groups, and is thought to be due primarily to glucose-associated osmotic pressure causing a dilutional effect.(238) This might explain some of the association with comorbidity, inflammation and albumin found by Szeto. (235)

Ethnicity is a strong risk factor for the development of type II diabetes, with white Caucasians having a lower risk than Asians, Hispanics and Afro-Caribbeans**.** (239) This is commensurate with our observation in the incident group of Koreans having higher glucose levels than the predominantly white Caucasian reference population and may partly explain the higher glucose levels in the study by Szeto et al. (235)

The peritoneal solute transport rate is one of the potential determinants of the systemic effects of dialysate glucose, through modification of glucose absorption. We found no effect, possibly because the difference in absorption was relatively minor in comparison to the total amount absorbed however this study used a dialysate/plasma creatinine ratio as the measure of peritoneal solute transport rate. The D/D0 glucose may be a better measure for this analysis but was not available.

Another important clinical question is whether it matters how the dialysate is prescribed. We tested for this by including the type of PD (APD vs CAPD) both as a main effect and an interaction and found no effect. This suggests that how the dialysate glucose is prescribed matters less than the amount that is prescribed.

Icodextrin does not affect glucose levels in diabetic PD patients (240) so, although the main effect of Icodextrin on glucose levels was not significant, we also tested for an interaction which was highly significant. For patients on Icodextrin the predicted glucose initially declined with increased DDG but subsequently rose. Indication bias could explain this, if an abnormality of glucose metabolism not formally diagnosed as diabetes was recognised and treated with Icodextrin usage and glucose minimisation. The same relationship between increased DDG and glucose levels found in patients not on Icodextrin could explain the subsequent rise in glucose levels with increased DDG for patients on Icodextrin.

The survival analysis has confirmed the results from Szeto et al, (235) with a significant unadjusted effect on mortality of plasma glucose levels, but we extended this into an adjusted analysis. In incident patients there is no significant independent effect on mortality but it remains unclear whether there is an effect in prevalent patients, when dialysate glucose has its greatest effect. That two studies have now shown an association between plasma glucose levels and unadjusted mortality rates appears to contradict a more recent finding from Szeto et al where MetS, no matter which definition was used, was not associated with mortality. (241) This apparent inconsistency may be explained by the limitations of applying diagnostic criteria used in the general population for MetS to the PD population.

That ethnically Chinese patients develop aspects of MetS during PD, and that this is associated with prolonged glucose exposure, was established in 2008 by Jiang et al, (185) but they found no association between glucose exposure and systemic levels. This was possibly as they correlated the most common dialysate glucose prescription during PD with subsequent glucose levels rather than

measuring the prescription in use at the time of glucose measurement. Our study used simultaneous measurements of dialysate glucose exposure and systemic levels to successfully demonstrate this association in a mix of British, Canadian and Korean prevalent patients. The difference in this association between incident and prevalent patients may be due to the sustained glucose loading prevalent patients have undergone but informative censoring could also theoretically explain this difference.

In the absence of larger studies using better markers of glucose metabolism, clear guidance on the safe dose of dialysate glucose is not possible, but this study provides more evidence in support of minimising dialysate glucose exposure and suggests that, particularly if larger glucose doses are being used, systemic glucose metabolism should be monitored.

Limitations of this study include evidence of digit preference in blood pressure measures and incomplete information on the presence of metabolic syndrome as the study was not primarily designed to investigate glucose metabolism. Glucose levels were measured locally although use of a multilevel model should account for this. The study did not contain sufficiently reliable information on automated PD regimes to investigate the effect of different regimes rather than total dialysate glucose exposure. Whilst the study used 10 centres from 3 countries a degree of selection bias might be present as the selected centres had better data quality. As an observational study, causality cannot be proven although the association between dialysate glucose and systemic levels would fulfil most of the Bradford-Hill criteria for causality.

Dialysate glucose load appears to have a major effect on systemic glucose levels and the effects are under-recognised. This should be factored in when prescribing peritoneal dialysate and increased awareness of the potential problems are necessary.

10 Conclusion

10.1 Findings So Far

10.1.1 Chapter 4

The previous literature did not contain clarity on the relative roles of peritoneal and systemic inflammation, both in relation to each other, and in relation to PSTR and patient survival. In Chapter 4 we have shown that the peritoneal membrane is inflamed, that this is mostly independent of systemic inflammation and that peritoneal inflammation is the strongest determinant of PSTR through IL-6. We have further shown that patient survival is directly affected by systemic and not peritoneal inflammation, although PSTR is still a determinant of patient survival, so peritoneal inflammation may have an indirect effect on patient survival. One important qualification to the above paragraph lies in the relationship between peritoneal and plasma IL-6, which was a consistent finding in all analyses.

10.1.2 Chapter 5

This chapter needs to be regarded as an exploratory analysis, partly as it was driven by the hypothesis that analysing changes with time in biological variables by using pre-specified arbitrary time points can lead to misleading results, and partly because it was using cross-sectional data to infer longitudinal change. Despite these misgivings, we replicated the previous finding of a sharp increase in PSTR in the first few weeks of PD, suggesting that, as a minimum, the early changes do reflect those of the PD population and are not due to informative censoring. This change was accompanied by an increase in systemic inflammation and in peritoneal IL-6. There was also an unexpected, but very consistent, finding of a fall in peritoneal inflammation over the subsequent 16 months.

10.1.3 Chapter 6

We have demonstrated that PD patients with subsequent EPS have a more inflamed peritoneum, that this is not evident when measuring the PSTR and that plasma IL-6 levels are also higher in this group

10.1.4 Chapter 7

In this chapter we replicated the finding that PSTR is not significantly different between EPS patients and time matched controls, but EPS patients do have a significantly poorer UF capacity and higher dialysate glucose requirements, which is likely to be due to increased fibrosis.

10.1.5 Chapter 8

Managing PD patients to minimise the risk of EPS, whilst optimising the length of time they can stay on their chosen modality is an important clinical principle, and this chapter describes the development of a prognostic model to aid in this.

10.1.6 Chapter 9

Whilst the preceding 3 chapters dealt with a local complication of PD, this chapter describes a novel feature of the systemic complications of PD where increased dialysate glucose loading impairs systemic glucose metabolism in non-diabetic patients.

10.2 Discussion

Having established in chapter 4 that the peritoneal membrane is inflamed, one of the key questions is ‘what is the significance of peritoneal inflammation’. There are a number of ways in which it could be significant described in the following hypotheses:

1 - Peritoneal inflammatory mediators are absorbed, partially driving the systemic inflammation associated with malnutrition, atherosclerosis and mortality that is common in dialysis patients

2 – Peritoneal inflammation drives fibrosis with a decrease in the osmotic conductance to glucose and the subsequent risks of UF failure, technique failure and EPS

3 – Peritoneal inflammation drives angiogenesis, and thereby is responsible for the increase in PSTR associated with long term PD, of relevance because increased PSTR still contributes to increased mortality

4 – The level of peritoneal inflammation could alter the susceptibility of patients to develop infectious peritonitis by altering local immune responses

Of these hypotheses, this thesis provides some supportive evidence for the first 2.

Taking the first hypothesis, chapters 3, 4 and 5 all provide strong evidence of an association between dialysate and plasma IL-6 levels. Furthermore, as shown in chapter 4, for almost all patients there is a steep diffusion gradient from dialysate to peritoneal capillary and assuming that IL-6 production occurs within the peritoneal membrane rather than in the dialysate, local concentrations should be even higher with a steeper gradient.

If EPS is considered, this is generally accepted to be an inflammatory process and that systemic inflammation is evident in many patients, strongly suggesting that, as is the case with other local inflammatory conditions that causes systemic inflammation (e.g. gout), local inflammatory mediators diffuse into the systemic circulation. In Chapter 6, we showed that local inflammation precedes the diagnosis of EPS but the systemic inflammation is also evident pre-diagnosis suggesting that systemic evidence of local inflammation occurs earlier than realised.

In Chapter 5, we showed that initiation of PD appears to induce a systemic inflammatory response. It is conceivable that this increase in inflammation is driven by absorption of dialysate contents such as GDPs but equally possible is the explanation that PD induces peritoneal inflammation (hence the rise in dialysate IL-6), which drives the increase in PSTR, and the IL-6 is absorbed and drives the increase in systemic inflammation.

As with all studies, there are caveats to this evidence (as discussed in the chapters), but the first hypothesis is one of the simplest explanations for the findings. The only other obvious rival hypothesis for the associations described would be a genotypic association where IL-6 alleles associated with increased IL-6 production cause an increase in both peritoneal and systemic production simultaneously, however this would not be such a convincing explanation for the findings in the case of EPS or for the changes occurring at the start of PD.

The supportive evidence for the second hypothesis is weaker but still suggestive. In chapter 7 we, as well as another group subsequently, (195) showed that patients with subsequent EPS have a decreased osmotic conductance to glucose compared with time matched controls, likely due to fibrosis. In chapter 5, we also showed that patients with subsequent EPS had worse peritoneal inflammation. The simplest explanation would be that the inflammation drives the fibrosis, as is thought to occur in many conditions, but this evidence is circumstantial.

10.3 Clinical Relevance

Although the peritoneum is inflamed during PD, this is not in itself clinically relevant so further work is required to establish the true clinical relevance. Most of the potentially relevant mechanisms by which peritoneal inflammation could have a clinical impact are discussed in the preceding section (10.2), but one further mechanism lies in the impact on PSTR. We have shown that this still predicts mortality in the most current study to examine this despite some articles suggesting that this concern is in the past, (242) and inflammation is the strongest determinant of this. There is a need to monitor current practice patterns as Icodextrin and APD have both been shown to improve the mortality associated with faster PSTR.

We have also demonstrated that decreased UF capacity, increased dialysate glucose requirements and increased dialysate inflammatory markers detect patients more likely to develop EPS. This suggests that it may be possible to use these factors to identify accurately the patients who require a

switch to HD to prevent EPS, without having to switch patients who would not have developed EPS. Once the prognostic model described in Chapter 8 has been fully developed and validated, data from the ongoing PD-CRAFT study will allow the testing of this theory.

By investigating the association between local and systemic effects of PD, we have shown that even in non-diabetic PD patients, increased dialysate glucose adversely affects systemic glucose metabolism. The obvious clinical implication of this is to minimise the dialysate glucose load, but this should not substantially alter current clinical management as there is already data linking dialysate glucose load to adverse effects in the peritoneal membrane (56), suggesting that dialysate glucose should be minimised. There is also data suggesting that glucose avoidance might be associated with harm if not managed carefully. (243) This study does however add further weight to need for new PD solutions not reliant on glucose and justifies further study, investigating the role of glucose loading in visceral fat accumulation and metabolic syndrome, and whether these effects are independently associated with mortality in an appropriately powered study.

10.4 Further Studies

Numerous further studies are possible, to investigate some of these findings. For studies that could be completed using the existing data possibilities include:

1 Investigating the possibility of using a combination of 24 hour UF and PET data to estimate the osmotic conductance to glucose, which could allow in-depth investigations of the timescale of fibrotic development, and mediators which play a role in it

2 Studying the role of angiogenesis. In particular, it would be possible to test whether baseline inflammation predicts future PSTR, and subsequently, whether angiogenic factors are predictive of future PSTR over and above the effect of inflammation

3 Whether peritonitis predicts an increase in long term inflammation, or inflammation predicts peritonitis, has not previously been studied with sufficient large patient numbers to detect

potentially subtle changes. To study this with GFS data would require some checking of the peritonitis data

4 One unexpected finding was the association between higher systolic BP and lower dialysate IL-6 levels. This requires an initial replication to validate the finding. If true, one hypothesis to explain it would be that a higher BP increases peritoneal perfusion, thereby increasing the clearance of dialysate cytokines to the circulation. This would be testable in further studies

5 Dialysate IL-6 and/or TNF-α could be added to the prognostic model of EPS in the validation phase of PD-CRAFT to see if this does improve the predictive power of existing factors

6 The existing repeated measures data in GFS could be used as a validation of the preliminary finding of a fall in peritoneal inflammation between months 2 and 18 of PD, as well as providing a more powerful model to detect the expected decrease in PSTR

7 A more detailed study to confirm the provisional finding that PD induces systemic inflammation with further clinical detail to delineate the relative roles of catheter insertion and dialysate

8 An animal model to test the hypothesis that peritoneal/dialysate IL-6 can induce systemic inflammmation

9 Development of a model to estimate the osmotic conductance to glucose from the existing data sets through inclusion of 24 hour UF rates combined with PET data, which would allow associations with fibrosis to be tested more directly

11 Appendices

11.1 Appendix A – Supplementary Analysis of Predictors of Dialysate IL-6

In Chapter 4, determinants of dialysate IL-6 concentrations were investigated. In the primary analysis, PSTR was excluded as this is highly likely to be an effect of IL-6, rather than a cause of high levels of IL-6, however there is potentially a complicated interaction with solution types. A fast PSTR will be treated with higher glucose concentrations and converting to using Icodextrin solutions, but higher glucose concentrations and the use of Icodextrin solutions are two of the main mechanisms by which PD may be inducing peritoneal inflammation. Table 11-1 shows a further analysis, using the same data and methodology as that for Table 4-5, but including PSTR as a predictor to attempt to distinguish an effect of solution type.

The inclusion of PSTR makes little difference to the overall results. The change in the predicted effect of dialysate glucose concentration makes the effect statistically insignificant in the incident patients, and it remains insignificant in the prevalent group. The effect of Icodextrin on dialysate IL-6 remains significant although the magnitude is attenuated. The p value for BMI in the prevalent patients drops to below 0.05, suggesting a possible effect of BMI on dialysate IL-6, but this is a secondary analysis so this must be viewed with significant caution.

This suggests that Icodextrin has an additional effect on peritoneal inflammation, over and above the general effect of PD. This is consistent with the existing literature. Martikainen reported a randomised, cross-over trial of 22 patients, where stable PD patients had 8 weeks of either nocturnal Icodextrin or daytime amino-acid dialysate, with 8 weeks washout, then 8 weeks of the other therapy.(51) Dialysate taken from the end of the nocturnal dwell at the start and end of each regime demonstrated that IL-6 rose with both Icodextrin and amino-acid dialysate, whilst Icodextrin was associated with an increase in dialysate TNF-α as well as plasma CRP.

Table 11-1: Predictors of Dialysate IL-6 Including PSTR

	Incident		Prevalent	
	Coefficient (95% CI)	**p value**	**Coefficient (95% CI)**	**p value**
Age (per decade)	**0.030 *** (0.007, 0.053)	0.01	**0.05 **** (0.02, 0.08)	0.003
BMI	0.003 (-0.005, 0.011)	0.4	**0.01 *** (0.001, 0.02)	0.03
APD usage	-0.03 (-0.18, 0.12)	0.7	-**0.01** * (-0.3, -0.003)	0.046
Systolic BP (per 10 mmHg)	-0.03 (-0.04, -0.01)	0.7	**-0.03 **** (-0.05, -0.007)	0.009
Male Gender	0.01 (-0.06, 0.09)	0.7	0.02 (-0.07, 0.11)	0.6
Duration of PD (per year)	-0.1 (-0.8, 0.6)	0.8	0.003 (-0.03, 0.03)	0.8
Biocompatible solution usage	0.01 (-0.07, 0.1)	0.8	0.13 (-0.001, 0.26)	0.052
Icodextrin usage	**0.2 **** (0.1, 0.3)	<0.001	**0.1 *** (0.02, 0.3)	0.03
Average Daily Glucose (per g/L)	0.009 (-0.001, 0.019)	0.08	-0.008 (-0.02, 0.005)	0.2
PSTR (per 0.1 D/P Cr)	**0.10** ** (0.07, 0.14)	<0.001	**0.15 **** (-0.11, 0.19)	<0.001
Dialysate TNF	**0.7 **** (0.4, 0.9)	<0.001	**0.6 **** (0.2, 0.9)	0.001
Dialysate IFN	0.04 (-0.08, 0.15)	0.5	-0.02 (-0.16, 0.13)	0.8
Plasma IL6	**0.3** ** (0.2, 0.4)	<0.001	**0.3 **** (0.1, 0.5)	0.004
Plasma TNF	-0.1 (-0.4, 0.1)	0.2	0.3 (-0.04, 0.6)	0.09
Plasma IFN	0.04 (-0.08, 0.2)	0.5	-0.1 (-0.3, 0.05)	0.2
Plasma IL1	-0.04 (-0.4, 0.3)	0.8	-0.04 (-0.4, 0.3)	0.8
Diabetes	-0.02 (-0.10, 0.06)	0.6	0.03 (-0.09, 0.16)	0.6
Comorbidity	0.02 (-0.02, 0.07)	0.3	-0.006 (-0.06, 0.05)	0.8
Residual Renal Function (per litre)	0.006 (-0.04, 0.06)	0.8	**-0.11 **** (-0.18, -0.03)	0.006
Korean	-0.1 (-0.3, 0.1)	0.4	-0.4 (-1.0, 0.2)	0.2

* p<0.05, **p<0.01

There has been one RCT study of dialysate markers with patients randomised to 12 months of a regime described as 'biocompatible' of Icodextrin with biocompatible dialysate and one amino acid dialysate or a regime of standard glucose-based dialysate.(55) The dialysate sample was taken from the overnight dwell of Icodextrin or standard glucose dialysate, with the 'biocompatible' group developing higher dialysate levels of IL-6, CA125 and adiponectin. A secondary analysis of the Balanz trial has shown that biocompatible dialysate had no effect on dialysate IL-6 levels, (244) compatible with the finding from the Global Fluid Study. Whether amino acid dialysate affects dialysate cytokine levels is not known but Icodextrin seems the most likely explanation for the increase in IL-6. Of note, the 'biocompatible' group in this study also had faster PSTR by the end of the study.

Moriishi reported a cohort study of 8 patients with dialysate white cell counts and IL-6 levels measured before and after switching a nocturnal dwell from 2.5% glucose to Icodextrin.(52) The white cell count rose with Icodextrin although IL-6 did not change. This study was very small and as a single cross-over non-randomised study, can only be considered as weak evidence.

Opatrna reported 33 patients allocated to a Physioneal only group or Physioneal with overnight Icodextrin based on whether the UF from a 4 hour PET was greater than or less than 400ml. The Icodextrin group had a faster baseline PSTR and had higher dialysate IL-6 levels but this study has significant indication bias.(53)

Taken in combination with the previous studies, our study shows that it is highly likely that Icodextrin increases peritoneal inflammation.

11.2 Appendix B - Assumption Checks for Chapter 4

All models were built after checking the variance inflation factor (VIF) of covariates using a maximum allowed VIF score of 5 and, where necessary, the correlation matrices.

11.2.1 Post-estimation Checks for Multilevel Models

The process for model checking is illustrated by the checks applied to the multilevel model of D/P Cr in incident patients, shown in Figure 11-1, Figure 11-2, Figure 11-3 and Figure 11-4. Level 1 residuals were checked for normality with a by-eye assessment of normal quantile plots, and transformations from the 'ladder-of-powers' applied to achieve normality as necessary. The leverage and influence residuals were checked with boxplots and extreme outliers identified. Individuals with these residuals were identified and the data checked and corrected. Where the data was clinically implausible and could not be corrected, the data was set to missing, but if the data was clinically plausible the data was retained. If the data was retained, a sensitivity analysis excluding this point was performed but the results at no point significantly changed the outcome of the model. The standardised residuals were also plotted against the predicted values of D/P Cr to check for homoscedasticity.

Figure 11-1: Patient Level Residual Normal Quantile Plot for Incident Model of D/P Cr

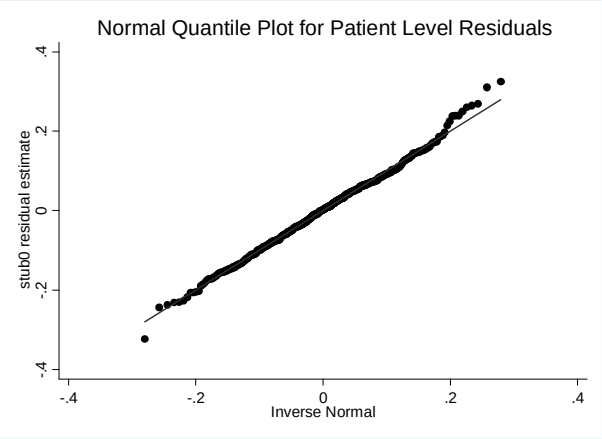


Figure 11-2: Leverage Residuals for Model of D/P Cr

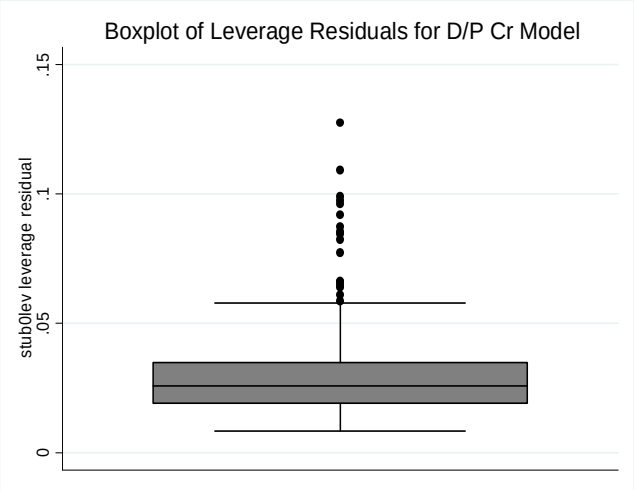


Figure 11-3: Boxplot of Influence Residuals for Incident Model of D/P Cr

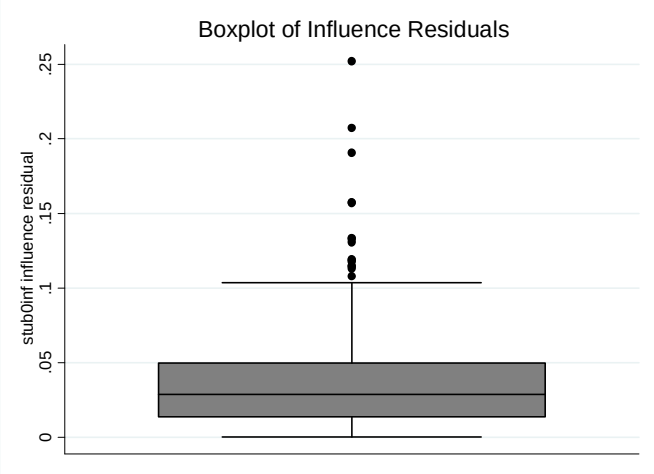
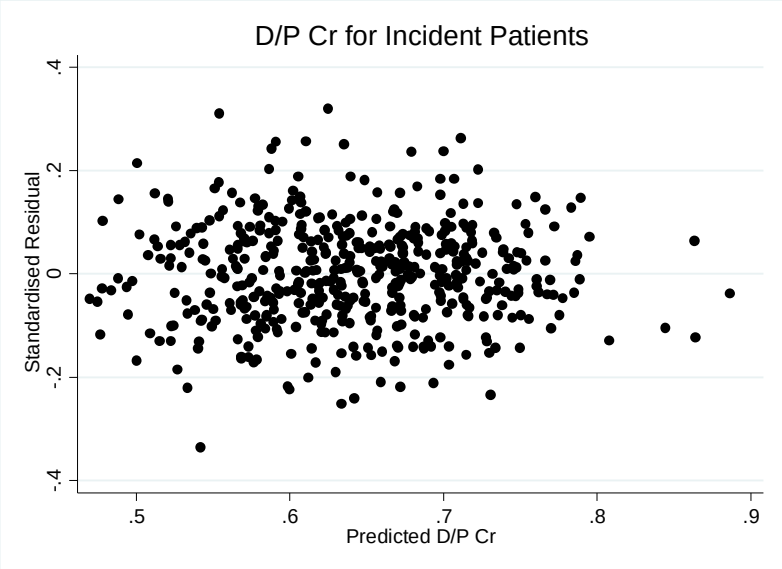


Figure 11-4: Homoscedasticity Check for D/P Cr Model in Incident Patients



11.2.2 Post Estimation Checks for Cox Models

All Cox models were checked for proportional hazards by linear regression of scaled Schoenfeld residuals on time, via the estat phtest within Stata. (245) The global test was used initially, and if the null hypothesis was rejected, individual covariates were tested and log-log plots run. None of the models presented in this thesis violated the non-proportional hazards.

11.3 Appendix C – Note on Statistical Tests in Chapter 7

Since publication of Chapter 7 as an original article, it has become clear that there may be more appropriate statistical tests as the t-tests used do not take account of clustering from case-control matching. One option would be conditional logistic regression, with the dependent variable a binary variable for case/control and the predictor variable the PSTR, UF capacity or glucose exposure. This design would require the same test to be repeated at multiple time points, so another option would be the same approach as that taken in Chapter 6 taking account of the repeated measures. This strategy would involve a 3 level model with the dependent variable one of the continuous variables (PSTR, UF capacity or Glucose exposure), with a random effect for measurements (level 1), for patients (level 2) and for case-control groups (level 3). Whether a patient is a case or control would then be a level 2 predictor variable.

The analysis in chapter 7 was the first one to completed, before the other chapters.

11.4 Appendix D – Additional Data checking for PDDB Data

Sense Checking

If PD episode record has a previous modality of CKD, there should be no PD episode with start date earlier

If the PD episode has finished, with a reason of peritonitis, there should be a peritonitis record within the last month

If the PD episode has finished, and the outcome is transplant, the reason should be transplant, and vice versa

If the PD episode has finished, and the outcome is died, the reason should be died, and vice versa

If the PD episode has finished, and the outcome is recovered, the reason should be recovered, and vice versa

If the PD episode has finished, and the reason is transferred, the outcome should be PD

If the PD episode has finished, and the reason is one of: peritonitis, adequacy, uf failure type I, uf failure type II, other technique failure, exit site infection, tunnel infection, patient choice, diagnosed eps, then the outcome should be HD

If the PD episode has finished, and the reason is withdrawal of treatment, the outcome should be died

If the outcome is 'died', there should be a date of death in the demographics data and it should be the same as PD finish date

If the blood test and the PET data are from the same day, the blood test creatinine and the PET plasma creatinine should be the same

If there is a date of death in the demographics data, there must be a PD finish date. In this situation, the reason in the PD episode data can be different to 'died' if the PD finish date is before the date of death

If the primary renal diagnosis is diabetic renal disease (code 80), the comorbidity data must include diabetes = true

If there is more than one comorbidity record, a comorbidity recorded in the earlier record must be recorded in the second record too

If PD regime type = APD, the sum of all bag volumes must be greater than or equal to the sum of all exchange volumes excluding the long dwell and extra fill exchange volumes

If PD regime type = APD, the PD adequacy overnight volume in must equal the sum of the bag volumes in PD regime

If PD regime type = APD, and long dwell type != dry day the long dwell volume in/out and urea/creatinine must be entered and the volume in (adequacy field) must = long dwell exchange volume (regime field) and vice versa (if long dwell=dry day the longdwell fields in adequacy must be null)

If urine volume = 0 the urine urea/creatinine/sodium/protein must all = null or 0

If PD regime type = CAPD, all PD adequacy volume outs must be within 75% of the respective volume in

If PD regime type = APD, the PD adequacy overnight volume out must be within 3000 of volume in

If blood test glucose>20 comorbidity diabetes = true

If PD regime fluid type = Baxter Dianeal, Baxter Physioneal or Fresenius type, Glucose strength !=0 & !=1.1

If PD regime fluid type = Baxter Extraneal or Nutrineal, Glucose strength = 0

11.5 Appendix E - Full Results for Models

11.5.1 Chapter 4 Models

11.5.1.1 Full Model for D/P Cr in Incident Patients

Log likelihood	429.9235
Deviance	-859.847

Table 11-2: Level Summary for Full Model of D/P Cr in Incident Patients

		Observations per Group		
Variable	Groups	Minimum	Maximum	Average
cent	10	4	151	48.9

Table 11-3: Coefficients for Full Model of D/P Cr in Incident Patients

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
age10	0.001414	0.003247	0.44	0.663	-0.0049499	0.0077785
bmi	-0.00234	0.001109	-2.11	0.035	-0.0045152	-0.0001689
typepd	-0.02171	0.02088	-1.04	0.298	-0.062634	0.0192122
bpsys10	0.004514	0.002185	2.07	0.039	0.000231	0.0087959
sex	0.022628	0.009969	2.27	0.023	0.0030892	0.042166
lengthpdmo~h	0.080375	0.025614	3.14	0.002	0.030172	0.1305775
lengthpdmo~2	-0.0198	0.008706	-2.27	0.023	-0.0368593	-0.0027308
biocomp	-0.00531	0.012429	-0.43	0.669	-0.02967	0.0190525
icodex	0.057619	0.014349	4.02	0	0.0294949	0.0857432
avdayglu	0.004889	0.001388	3.52	0	0.0021684	0.0076094
logdil6	0.082844	0.011918	6.95	0	0.0594861	0.1062017
logdtnf	0.036039	0.03333	1.08	0.28	-0.0292867	0.1013653
logdifn	-0.00945	0.015995	-0.59	0.555	-0.0407973	0.0219013
logpil6	-0.02146	0.018353	-1.17	0.242	-0.0574324	0.0145091
logptnf	0.021147	0.033243	0.64	0.525	-0.0440076	0.086301
logpifn	-0.00875	0.016738	-0.52	0.601	-0.0415532	0.0240594
logpil1	0.024295	0.044026	0.55	0.581	-0.061995	0.110585
diab	0.023716	0.011466	2.07	0.039	0.0012436	0.0461889
comorbscore	0.000455	0.006123	0.07	0.941	-0.0115458	0.0124553
urinevolli~e	0.025861	0.006947	3.72	0	0.0122455	0.0394773
korean	0.085472	0.038631	2.21	0.027	0.0097569	0.1611876
cons	0.393873	0.071954	5.47	0	0.252845	0.5349006

Table 11-4: Variance Estimates for Full Model of D/P Cr in Incident Patients

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 2: cent	var(cons)	0.002652	0.00134	2.63E-05	0.0052783
Level 1: id	var(cons)	0.009609	0.000621	0.008392	0.0108254

11.5.1.2 Random Intercept Model for D/P Cr in Incident Patients

Log likelihood	412.817
Deviance	-825.634

Table 11-5: Level Summary for Random Intercept Multilevel Model of D/P Cr in Incident Patients

		Observations per Group		
Variable	Groups	Minimum	Maximum	Average
cent	10	7	154	56.7

Table 11-6: Coefficients for Random Intercept Model of D/P Cr in Incident Patients

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
cons	0.699555	0.015536	45.03	0	0.669106	0.730004

Table 11-7: Variance Estimates for Random Intercept Model of D/P Cr in Incident Patients

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 2: cent	var(cons)	0.002008	0.001066	-8.2E-05	0.004098
Level 1: id	var(cons)	0.013169	0.000789	0.011623	0.014715

11.5.1.3 Full Model for D/P Cr in Prevalent Patients

Log likelihood	299.3478
Deviance	-598.696

Table 11-8: Level Summary for Full Model of D/P Cr in Prevalent Patients

		Observations per Group		
Variable	Groups	Minimum	Maximum	Average
cent	10	3	87	32.5

Table 11-9: Coefficients for Full Model of D/P Cr in Prevalent Patients

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
age10	-0.00268	0.004006	-0.67	0.504	-0.01053	0.005173
bmi	-0.00105	0.001261	-0.83	0.404	-0.00352	0.00142
typepd	-0.01294	0.017744	-0.73	0.466	-0.04772	0.021839
bpsys10	0.002195	0.002742	0.8	0.423	-0.00318	0.00757
sex	0.021195	0.010888	1.95	0.052	-0.00014	0.042535
lengthpdyear	0.010941	0.00352	3.11	0.002	0.004043	0.01784
biocomp	-0.03871	0.015792	-2.45	0.014	-0.06966	-0.00776
icodex	0.034497	0.015369	2.24	0.025	0.004375	0.064619
avdayglu	0.003523	0.001564	2.25	0.024	0.000458	0.006589
logdil6	0.087526	0.012393	7.06	0	0.063236	0.111816
logdtnf	-0.00243	0.043396	-0.06	0.955	-0.08749	0.082621
logdifn	0.008418	0.017438	0.48	0.629	-0.02576	0.042597
logpil6	-0.00257	0.023682	-0.11	0.914	-0.04899	0.043844
logptnf	-0.0589	0.036095	-1.63	0.103	-0.12965	0.011843
logpifn	-0.0059	0.020211	-0.29	0.771	-0.04551	0.033718
logpil1	-0.01301	0.045966	-0.28	0.777	-0.1031	0.077082
diab	0.002982	0.015367	0.19	0.846	-0.02714	0.033101
comorbscore	0.003173	0.006712	0.47	0.636	-0.00998	0.016329
urinevolli~e	0.021261	0.009226	2.3	0.021	0.003179	0.039343
korean	0.055787	0.028009	1.99	0.046	0.00089	0.110685
cons	0.577935	0.068056	8.49	0	0.444547	0.711322

Table 11-10: Variance Estimates for Full Model of D/P Cr in Prevalent Patients

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 2: cent	var(cons)	0.001147	0.000705	-0.00023	0.002528
Level 1: id	var(cons)	0.008881	0.000707	0.007495	0.010266

11.5.1.4 Random Intercept Model for D/P Cr in Prevalent Patients

Log likelihood	292.7065
Deviance	-585.413

Table 11-11: Level Summary for Random Intercept Model of D/P Cr in Prevalent Patients

		Observations per Group		
Variable	Groups	Minimum	Maximum	Average
cent	10	4	105	38.1

Table 11-12: Coefficients for Random Intercept Model of D/P Cr in Prevalent Patients

dpcr	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
cons	0.689887	0.01583	43.58	0	0.65886	0.720914

Table 11-13: Variance Estimates for Random Intercept Model of D/P Cr in Prevalent Patients

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 2: cent	var(cons)	0.001945	0.001107	-0.00022	0.004114
Level 1: id	var(cons)	0.012038	0.000883	0.010307	0.013769

11.5.1.5 Full Model for Dialysate IL-6 in Incident Patients

Log likelihood	-231.31
Deviance	462.6205

Table 11-14: Level Summary for Full Model of Dialysate IL-6 in Incident Patients

		Observations per Group		
Variable	Groups	Minimum	Maximum	Average
cent	10	5	151	49.7

Table 11-15: Coefficients for Full Model of Dialysate IL-6 in Incident Patients

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
age10	0.037885	0.0123141	3.08	0.002	0.01375	0.062021
bmi	0.000915	0.0042185	0.22	0.828	-0.00735	0.009183
typepd	-0.05747	0.078543	-0.73	0.464	-0.21141	0.096472
bpsys10	-0.01865	0.0082357	-2.26	0.024	-0.03479	-0.00251
sex	0.038809	0.0381828	1.02	0.309	-0.03603	0.113646
lengthpdyear	0.143455	0.3549725	0.4	0.686	-0.55228	0.839188
biocomp	0.000568	0.046829	0.01	0.99	-0.09122	0.092351
icodex	0.303538	0.0522712	5.81	0	0.201089	0.405988
avdayglu	0.014247	0.0051945	2.74	0.006	0.004066	0.024428
logdtnf	0.800452	0.119087	6.72	0	0.567046	1.033858
logdifn	0.005593	0.0609767	0.09	0.927	-0.11392	0.125105
logpil6	0.317591	0.0689031	4.61	0	0.182543	0.452638
logptnf	-0.18886	0.1244842	-1.52	0.129	-0.43285	0.055121
logpifn	0.06221	0.0635425	0.98	0.328	-0.06233	0.186751
logpil1	-0.01272	0.1687628	-0.08	0.94	-0.34349	0.318044
diab	0.009814	0.0439606	0.22	0.823	-0.07635	0.095975
comorbscore	0.015673	0.0233533	0.67	0.502	-0.0301	0.061444
urinevolli~e	0.032694	0.0264494	1.24	0.216	-0.01915	0.084534
korean	-0.02275	0.1075738	-0.21	0.832	-0.23359	0.188088
cons	0.341007	0.2641004	1.29	0.197	-0.17662	0.858634

Table 11-16: Variance Estimates for Full Model of Dialysate IL-6 in Incident Patients

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 2: cent	var(cons)	0.0177819	0.0099784	-0.00178	0.037339
Level 1: id	var(cons)	0.1433934	0.0091857	0.12539	0.161397

11.5.1.6 Random Intercept Model for Dialysate IL-6 in Incident Patients

Table 11-17: Level Summary for Random Intercept Model for Dialysate IL-6 in Incident Patients

		Observations per Group		
Variable	Groups	Minimum	Maximum	Average
cent	10	7	153	56.3

Log likelihood	-363.436
Deviance	726.8721

Table 11-18: Coefficients for Random Intercept Model for Dialysate IL-6 in Incident Patients

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
cons	0.800349	0.07826	10.23	0	0.646963	0.953734

Table 11-19: Variance Estimates for Random Intercept Model for Dialysate IL-6 in Incident Patients

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 2: cent	var(cons)	0.054453	0.02702	0.001496	0.10741
Level 1: id	var(cons)	0.20367	0.012249	0.179662	0.227677

11.5.1.7 Full Model for Dialysate IL-6 in Prevalent Patients

Table 11-20: Level Summary for Full Model of Dialysate IL-6 in Prevalent Patients

		Observations per Group		
Variable	Groups	Minimum	Maximum	Average
cent	10	3	88	32.8

Log likelihood	-188.728
Deviance	377.4554

Table 11-21: Coefficients for Full Model of Dialysate IL-6 in Prevalent Patients

logdil6	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
age10	0.051059	0.017508	2.92	0.004	0.016744	0.085373
bmi	0.010918	0.005575	1.96	0.05	-9.09E-06	0.021845
typepd	-0.17697	0.077524	-2.28	0.022	-0.32892	-0.02503
bpsys10	-0.03106	0.012006	-2.59	0.01	-0.05459	-0.00753
sex	0.059505	0.048081	1.24	0.216	-0.03473	0.153742
lengthpdyear	0.023439	0.015612	1.5	0.133	-0.00716	0.054038
biocomp	0.076668	0.070251	1.09	0.275	-0.06102	0.214357
icodex	0.219818	0.067272	3.27	0.001	0.087968	0.351668
avdayglu	-0.00238	0.006963	-0.34	0.732	-0.01603	0.011263
logdtnf	0.666616	0.189182	3.52	0	0.295825	1.037407
logdifn	0.002529	0.077119	0.03	0.974	-0.14862	0.15368
logpil6	0.326744	0.103813	3.15	0.002	0.123274	0.530214
logptnf	0.181172	0.163303	1.11	0.267	-0.13889	0.501239
logpifn	-0.1419	0.088986	-1.59	0.111	-0.31631	0.032507
logpil1	-0.06615	0.203804	-0.32	0.745	-0.4656	0.333294
diab	0.035793	0.068137	0.53	0.599	-0.09775	0.169339
comorbscore	-0.00114	0.029785	-0.04	0.969	-0.05952	0.057237
urinevolli~e	-0.08838	0.040686	-2.17	0.03	-0.16813	-0.00864
korean	-0.13804	0.149183	-0.93	0.355	-0.43043	0.154353
cons	0.552408	0.303952	1.82	0.069	-0.04333	1.148142

Table 11-22: Variance Estimates for Full Model of Dialysate IL-6 in Prevalent Patients

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 2: cent	var(cons)	0.036681	0.020476	-0.00345	0.076814
Level 1: id	var(cons)	0.175226	0.013889	0.148005	0.202447

11.5.1.8 Random Intercept Model for Dialysate IL-6 in Prevalent Patients

Table 11-23: Level Summary for Random Intercept Model of Dialysate IL-6 in Prevalent Patients

		Observations per Group		
Variable	Groups	Minimum	Maximum	Average
cent	10	4	103	37.8

Log likelihood	-285.468
Deviance	570.935

Table 11-24: Coefficients for Random Intercept Model of Dialysate IL-6 in Prevalent Patients

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
cons	0.848769	0.090813	9.35	0	0.670779	1.026759

Table 11-25: Variance Estimates for Random Intercept Model of Dialysate IL-6 in Prevalent Patients

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 2: cent	var(cons)	0.069849	0.036332	-0.00136	0.141057
Level 1: id	var(cons)	0.250393	0.018456	0.214219	0.286567

11.5.1.9 Full Model for Plasma IL-6 in Incident Patients

Table 11-26: Level Summary of Full Model of Plasma IL-6 in Incident Patients

		Observations per Group		
Variable	Groups	Minimum	Maximum	Average
cent	10	5	151	49.7

Log likelihood	-5.8023958
Deviance	11.604792

Table 11-27: Coefficients for Full Model of Plasma IL-6 in Incident Patients

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
age10	0.0229046	0.007897	2.9	0.004	0.007427	0.038382
bmi	0.0001357	0.002681	0.05	0.96	-0.00512	0.005389
typepd	0.0406607	0.049519	0.82	0.412	-0.0564	0.137717
bpsys10	0.0031928	0.005267	0.61	0.544	-0.00713	0.013517
sex	0.0466678	0.024353	1.92	0.055	-0.00106	0.094398
lengthpdyear	-0.1937804	0.209258	-0.93	0.354	-0.60392	0.216357
biocomp	0.0030713	0.029127	0.11	0.916	-0.05402	0.060159
icodex	0.0432039	0.033363	1.29	0.195	-0.02219	0.108594
avdayglu	0.0026081	0.003206	0.81	0.416	-0.00368	0.008892
logdil6	0.1251345	0.027721	4.51	0	0.070803	0.179466
logdtnf	-0.1564878	0.077363	-2.02	0.043	-0.30812	-0.00486
logdifn	0.0724148	0.038314	1.89	0.059	-0.00268	0.147509
logptnf	0.3860026	0.073364	5.26	0	0.242212	0.529793
logpifn	0.0490346	0.040691	1.21	0.228	-0.03072	0.128786
logpil1	0.2105526	0.106831	1.97	0.049	0.001169	0.419937
diab	-0.0482247	0.028114	-1.72	0.086	-0.10333	0.006877
comorbscore	0.0482531	0.01482	3.26	0.001	0.019206	0.0773
urinevolli~e	-0.0182947	0.016753	-1.09	0.275	-0.05113	0.014541
korean	0.0338079	0.04028	0.84	0.401	-0.04514	0.112756
cons	-0.386214	0.161469	-2.39	0.017	-0.70269	-0.06974

Table 11-28: Variance Estimates for Full Model of Plasma IL-6 in Incident Patients

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 2: cent	var(cons)	0.001188	0.001132	-0.00103	0.003407
Level 1: id	var(cons)	0.059188	0.003787	0.051764	0.066611

11.5.1.10 Random Intercept Model for Plasma IL-6 in Incident Patients

Table 11-29: Level Summary of Random Intercept Model of Plasma IL-6 in Incident Patients

		Observations per Group		
Variable	Groups	Minimum	Maximum	Average
cent	10	6	153	55.7

Log likelihood	-81.0924
Deviance	162.1849

Table 11-30: Coefficients for Random Intercept Model of Plasma IL-6 in Incident Patients

logdil6	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
cons	0.413245	0.02762	14.96	0	0.359111	0.46738

Table 11-31: Variance Estimates for Random Intercept Model of Plasma IL-6 in Incident Patients

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 2: cent	var(cons)	0.005443	0.003327	-0.00108	0.011964
Level 1: id	var(cons)	0.076357	0.004614	0.067313	0.085401

11.5.1.11 Full Model for Plasma IL-6 for Prevalent Patients

Table 11-32: Level Summary for Full Model of Plasma IL-6 in Prevalent Patients

		Observations per Group		
Variable	Groups	Minimum	Maximum	Average
cent	10	3	88	32.8

Log likelihood	24.86937
Deviance	-49.7387

Table 11-33: Coefficients for Full Model of Plasma IL-6 in Prevalent Patients

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
age10	0.008086	0.009305	0.87	0.385	-0.01015	0.026324
bmi	0.004496	0.002936	1.53	0.126	-0.00126	0.01025
typepd	-0.00401	0.040933	-0.1	0.922	-0.08424	0.076214
bpsys10	-0.00723	0.006377	-1.13	0.257	-0.01973	0.005265
sex	0.023522	0.02536	0.93	0.354	-0.02618	0.073226
lengthpdyear	0.014752	0.008114	1.82	0.069	-0.00115	0.030655
biocomp	-0.01799	0.036581	-0.49	0.623	-0.08969	0.053708
icodex	-0.01348	0.035513	-0.38	0.704	-0.08309	0.056123
avdayglu	-0.00191	0.003604	-0.53	0.596	-0.00898	0.005151
logdil6	0.086009	0.028178	3.05	0.002	0.030781	0.141237
logdtnf	-0.14635	0.099532	-1.47	0.141	-0.34143	0.048732
logdifn	0.052694	0.040279	1.31	0.191	-0.02625	0.131639
logptnf	0.404238	0.076752	5.27	0	0.253808	0.554669
logpifn	0.142358	0.046362	3.07	0.002	0.051489	0.233227
logpil1	0.317477	0.105838	3	0.003	0.110038	0.524916
diab	0.075107	0.035636	2.11	0.035	0.005263	0.144951
comorbscore	0.020197	0.015715	1.29	0.199	-0.0106	0.050999
urinevolli~e	-0.00771	0.021514	-0.36	0.72	-0.04988	0.034453
korean	-0.03236	0.047242	-0.69	0.493	-0.12495	0.060231
cons	-0.2496	0.154417	-1.62	0.106	-0.55225	0.053055

Table 11-34: Variance Estimates for Full Model of Plasma IL-6 in Prevalent Patients

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 2: cent	var(cons)	0.002167	0.001813	-0.00139	0.00572
Level 1: id	var(cons)	0.049131	0.003888	0.04151	0.056751

11.5.1.12 Random Intercept Model for Plasma IL-6 in Prevalent Patients

Table 11-35: Level Summary for Random Intercept Model of Plasma IL-6 in Prevalent Patients

		Observations per Group		
Variable	Groups	Minimum	Maximum	Average
cent	10	3	105	37.9

Log likelihood	-42.4198
Deviance	84.83951

Table 11-36: Coefficients for Random Intercept Model of Plasma IL-6 in Prevalent Patients

logdil6	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
cons	0.40487	0.013901	29.12	0	0.377624	0.432116

Table 11-37: Variance Estimates for Random Intercept Model of Plasma IL-6 in Prevalent Patients

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 2: cent	var(cons)	0	0	0	0
Level 1: id	var(cons)	0.073239	0.00532	0.062811	0.083667

11.5.1.13 Cox Model for Incident Patients

No. of subjects = 503 Number of obs = 503

No. of failures = 225

Time at risk = 31302.1109

Wald chi2(15) = 208.91

Log pseudolikelihood = -725.68389 Prob > chi2 = 0.0000

Table 11-38: Coefficients for Cox Survival Model in Incident Patients

	Haz. Ratio	Robust SE	z	P>z	95% Confidence Interval	
logdtnf	0.963213	0.52718	-0.07	0.945	0.329494	2.815766
logdil6	0.932663	0.163013	-0.4	0.69	0.662141	1.313709
logdifn	1.190621	0.32012	0.65	0.516	0.702931	2.016667
logpil1	0.577001	0.393083	-0.81	0.42	0.151808	2.193103
logptnf	3.412823	1.734047	2.42	0.016	1.260725	9.238622
logpil6	2.161273	0.62147	2.68	0.007	1.230122	3.797265
logpifn	0.85546	0.246682	-0.54	0.588	0.486122	1.505406
age	1.060887	0.007865	7.97	0	1.045584	1.076414
sex	0.933476	0.146787	-0.44	0.662	0.68589	1.270435
comorbscore	1.684828	0.134537	6.53	0	1.440738	1.97027
urinevolli~e	0.951494	0.110534	-0.43	0.669	0.757745	1.194784
lengthpdmo~h	1.046634	0.145613	0.33	0.743	0.79684	1.374732
alb	0.942203	0.015974	-3.51	0	0.911409	0.974038
dpcr10	1.093906	0.063859	1.54	0.124	0.97564	1.226508
bmi	1.009792	0.020963	0.47	0.639	0.969531	1.051725

11.5.1.14 Cox Model for Prevalent Patients

Stratified Cox regr. -- no ties

No. of subjects = 345 Number of obs = 345

No. of failures = 171

Time at risk = 680973

Wald chi2(15) = 195.02

Log pseudolikelihood = -516.0168 Prob > chi2 = 0.0000

Table 11-39: Coefficients for Cox Survival Model in Prevalent Patients

	Haz. Ratio	Robust SE	z	P>z	95% Confidence Interval	
logdtnf	0.90839	0.641631	-0.14	0.892	0.227528	3.62668
logdil6	0.958582	0.194264	-0.21	0.835	0.644358	1.426039
logdifn	1.195768	0.371555	0.58	0.565	0.650365	2.198551
logpil1	0.539634	0.326887	-1.02	0.309	0.164618	1.768973
logptnf	2.099505	1.42828	1.09	0.276	0.553413	7.964968
logpil6	2.685174	1.005032	2.64	0.008	1.289368	5.592012
logpifn	1.19547	0.373936	0.57	0.568	0.647572	2.206933
age	1.057003	0.007322	8	0	1.042749	1.071451
sex	1.28113	0.218211	1.45	0.146	0.917509	1.788859
comorbscore	1.368792	0.101122	4.25	0	1.184276	1.582055
urinevolli~e	0.648289	0.09722	-2.89	0.004	0.483192	0.869797
lengthpdyear	1.13504	0.05 1746	2.78	0.005	1.038018	1.241131
alb	0.988001	0.020798	-0.57	0.566	0.948068	1.029617
dpcr10	1.188856	0.103022	2	0.046	1.003153	1.408937
bmi	1.009726	0.017329	0.56	0.573	0.976327	1.044267

11.5.2 Chapter 5 Models

11.5.2.1 Full Model for Plasma TNF-α

Table 11-40: Level Summary for Full Model of Plasma TNF-alpha in All Patients

		Observations per Group		
Variable	Groups	Minimum	Maximum	Average
cent	10	20	242	83.1

Log likelihood	456.928
Deviance	-913.856

Table 11-41: Coefficients for Full Model of Plasma TNF alpha in All Patients

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
age	-0.00021	0.000348	-0.61	0.545	-0.00089	0.000471
bmi	-0.00106	0.001151	-0.92	0.358	-0.00331	0.001198
typepd	0.017009	0.018209	0.93	0.35	-0.01868	0.052698
bpsys	-3.1E-05	0.000235	-0.13	0.894	-0.00049	0.000429
sex	0.01656	0.010148	1.63	0.103	-0.00333	0.03645
lengthpdyear	-0.00149	0.004065	-0.37	0.714	-0.00946	0.006479
biocomp	-0.02974	0.013194	-2.25	0.024	-0.0556	-0.00388
icodex	-0.00945	0.014322	-0.66	0.509	-0.03752	0.018621
avdayglu	3.727595	1.429484	2.61	0.009	0.925857	6.529333
logdil6	-0.00813	0.011874	-0.68	0.493	-0.0314	0.01514
logdtnf	0.050495	0.035452	1.42	0.154	-0.01899	0.11998
logdifn	-0.02318	0.016101	-1.44	0.15	-0.05473	0.00838
logpil6	0.142185	0.019098	7.45	0	0.104755	0.179616
logpifn	0.179527	0.016672	10.77	0	0.146851	0.212202
logpil1	0.075337	0.044142	1.71	0.088	-0.01118	0.161854
diab	0.01268	0.012694	1	0.318	-0.0122	0.037559
comorbscore	-0.01038	0.006259	-1.66	0.097	-0.02265	0.001887
urinevol	-3.3E-05	7.52E-06	-4.37	0	-4.8E-05	-1.8E-05
koreanvsno~n	-0.01501	0.074106	-0.2	0.839	-0.16025	0.130236
cons	0.926885	0.067334	13.77	0	0.794913	1.058856

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 2: cent	var(cons)	0.011101	0.0051317	0.001043	0.021159
Level 1: id	var(cons)	0.018659	0.0009209	0.016854	0.020464

11.5.3 Chapter 6 Results

11.5.3.1 Full Model for Dialysate IL-6 in EPS Case-Control Study

Table 11-42: Level Summary of Full Model of Dialysate IL-6 in EPS Case Control Study

		Observations per Group		
Level Variable	Groups	Minimum	Maximum	Average
casecontro~r	11	3	23	13.5
id	38	1	9	3.9

Log likelihood	-237.637
Deviance	475.2746

Table 11-43: Coefficients for Full Model of Dialysate IL-6 in EPS Case Control Study

logdil6	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
eps	0.792009	0.390661	2.03	0.043	0.026327	1.55769
ttillendyear	0.272882	0.074029	3.69	0	0.127788	0.417976
ageatpdfin~h	0.009291	0.011865	0.78	0.434	-0.01396	0.032545
cons	2.486986	0.768855	3.23	0.001	0.980058	3.993915

Table 11-44: Variance Estimates for Full Model of Dialysate IL-6 in EPS Case Control Study

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 3: casecontrolnumber	var(cons)	0.162202	0.218017	-0.2651	0.589507
Level 2: id	var(cons)	0.700469	0.288604	0.134816	1.266121
Level 1: samplenumber	var(cons)	1.041903	0.139339	0.768803	1.315003

11.5.3.2 Full Model for Dialysate TNF-α in EPS Case-Control Study

Table 11-45: Level Summary for Full Model of Dialysate TNF alpha in EPS Case Control Study

Level Variable	Groups	Observations per Group Minimum	Maximum	Average
casecontro~r	11	3	23	13.5
id	38	1	9	3.9

Log likelihood	-139.532
Deviance	279.0647

Table 11-46: Coefficients for Full Model of Dialysate TNF alpha in EPS Case Control Study

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
eps	0.642222	0.209165	3.07	0.002	0.232266	1.052177
ttillendyear	0.048472	0.038028	1.27	0.202	-0.02606	0.123006
ageatpdfin~h	0.019248	0.006046	3.18	0.001	0.007398	0.031098
cons	-0.67483	0.385964	-1.75	0.08	-1.43131	0.081647

Table 11-47: Variance Estimates for Full Model of Dialysate TNF alpha in EPS Case Control Study

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 3: casecontrolnumber	var(cons)	0.006949	0.047776	-0.08669	0.100588
Level 2: id	var(cons)	0.212745	0.08412	0.047873	0.377617
Level 1: samplenumber	var(cons)	0.277613	0.037111	0.204878	0.350349

11.5.3.3 Full Model for Dialysate IFN-γ in EPS Case-Control Study

Table 11-48: Level Summary for Full Model of Dialysate IFN gamma in EPS Case Control Study

Level Variable	Groups	Observations per Group		
		Minimum	Maximum	Average
casecontro~r	11	3	23	13.5
id	38	1	9	3.9

Log likelihood	-221.075
Deviance	442.1491

Table 11-49: Coefficients for Full Model of Dialysate IFN gamma in EPS Case Control Study

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
eps	0.616639	0.343559	1.79	0.073	-0.05672	1.290003
ttillendyear	0.085193	0.066258	1.29	0.199	-0.04467	0.215057
ageatpdfin~h	0.015503	0.010558	1.47	0.142	-0.00519	0.036196
cons	0.345255	0.687128	0.5	0.615	-1.00149	1.692

Table 11-50: Variance Estimates for Full Model of Dialysate IFN gamma in EPS Case Control Study

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 3: casecontrolnumber	var(cons)	0.161688	0.183069	-0.19712	0.520496
Level 2: id	var(cons)	0.528714	0.221822	0.093952	0.963476
Level 1: samplenumber	var(cons)	0.835408	0.111696	0.616488	1.054329

11.5.3.4 Full Model for Dialysate IL-1β in EPS Case-Control Study

Table 11-51: Level Summary for Full Model of Dialysate IL-1 beta in EPS Case Control Study

Level Variable	Groups	Observations per Group		
		Minimum	Maximum	Average
casecontro~r	11	3	23	13.5
id	38	1	9	3.9

Table 11-52: Coefficients for Full Model of IL-1 beta in EPS Case Control Study

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
eps	1.06241	0.595879	1.78	0.075	-0.10549	2.230312
ttillendyear	0.192594	0.141293	1.36	0.173	-0.08433	0.469522
ageatpdfin~h	0.02212	0.017296	1.28	0.201	-0.01178	0.056019
cons	-0.94932	1.104751	-0.86	0.39	-3.1146	1.215951

Table 11-53: Variance Estimates for Full Model of Dialysate IL-1 beta in EPS Case Control Study

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 3: casecontrolnumber	var(cons)	0.22239	0.42245	-0.6056	1.050377
Level 2: id	var(cons)	0.975642	0.601962	-0.20418	2.155466

11.5.3.5 Full Model for Plasma IL-6 in EPS Case-Control Study

Table 11-54: Level Summary for Full Model of Plasma IL-6 in EPS Case Control Study

		Observations per Group		
Level Variable	Groups	Minimum	Maximum	Average
casecontro~r	11	3	23	11.7
id	38	1	8	3.4

Log likelihood	-116.596
Deviance	233.1918

Table 11-55: Coefficients for Full Model of Plasma IL-6 in EPS Case Control Study

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
eps	0.420961	0.181537	2.32	0.02	0.065156	0.776766
ttillendyear	0.13184	0.039359	3.35	0.001	0.054698	0.208982
ageatpdfin~h	0.01587	0.005361	2.96	0.003	0.005363	0.026377
cons	0.384441	0.342016	1.12	0.261	-0.2859	1.05478

Table 11-56: Variance Estimates for Full Model of Plasma IL-6 in EPS Case Control Study

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 3: casecontrolnumber	var(cons)	0.019084	0.042471	-0.06416	0.102325
Level 2: id	var(cons)	0.130592	0.064869	0.003451	0.257733
Level 1: samplenumber	var(cons)	0.274992	0.040177	0.196248	0.353737

11.5.3.6 Full Model for Plasma TNF-α in EPS Case-Control Study

Table 11-57: Level Summary for Full Model of Plasma TNF alpha in EPS Case Control Study

Level Variable	Groups	Observations per Group Minimum	Maximum	Average
casecontro~r	11	3	23	11.7
id	38	1	8	3.4

Log likelihood	-68.1586
Deviance	136.3172

Table 11-58: Coefficients for Full Model of Plasma TNF alpha in EPS Case Control Study

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
eps	0.13261	0.131657	1.01	0.314	-0.12543	0.390653
ttillendyear	0.045348	0.026781	1.69	0.09	-0.00714	0.097838
ageatpdfin~h	0.009801	0.003874	2.53	0.011	0.002209	0.017393
cons	1.888265	0.246582	7.66	0	1.404973	2.371558

Table 11-59: Variance Estimates for Full Model of Plasma TNF alpha in EPS Case Control Study

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 3: casecontrolnumber	var(cons)	0.00733	0.021342	-0.0345	0.049159
Level 2: id	var(cons)	0.077533	0.034553	0.00981	0.145255
Level 1: samplenumber	var(cons)	0.122544	0.017901	0.087459	0.157628

11.5.3.7 Full Model for Plasma IFN-γ in EPS Case-Control Study

Table 11-60: Level Summary for Full Model of Plasma IFN gamma in EPS Case Control Study

Level Variable	Groups	Observations per Group Minimum	Maximum	Average
casecontro~r	11	3	23	11.7
id	38	1	8	3.4

Log likelihood	-148.457
Deviance	296.9133

Table 11-61: Coefficients for Full Model of Plasma IFN gamma in EPS Case Control Study

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
eps	-0.29641	0.199213	-1.49	0.137	-0.68686	0.094042
ttillendyear	0.11943	0.050042	2.39	0.017	0.02135	0.217511
ageatpdfin~h	0.013761	0.006547	2.1	0.036	0.000929	0.026593
cons	0.759605	0.448845	1.69	0.091	-0.12012	1.639325

Table 11-62: Variance Estimate for Full Model of Plasma IFN gamma in EPS Case Control Study

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 3: casecontrolnumber	var(cons)	0.226471	0.13413	-0.03642	0.48936
Level 2: id	var(cons)	0.108408	0.073452	-0.03556	0.252372
Level 1: samplenumber	var(cons)	0.444058	0.064334	0.317965	0.57015

11.5.3.8 Full Model for Plasma IL-1β in EPS Case-Control Study

Table 11-63: Level Summary for Full Model of Plasma IL-1 beta in EPS Case Control Study

Level Variable	Groups	Observations per Group		
		Minimum	Maximum	Average
casecontro~r	11	3	23	11.7
id	38	1	8	3.4

Table 11-64: Coefficients for Full Model of Plasma IL-1 beta in EPS Case Control Study

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
eps	0.658029	0.669498	0.98	0.326	-0.65416	1.97022
ttillendyear	-0.20968	0.173924	-1.21	0.228	-0.55056	0.131206
ageatpdfin~h	-0.02309	0.020668	-1.12	0.264	-0.0636	0.017414
cons	1.7807	1.338543	1.33	0.183	-0.8428	4.404196

Table 11-65: Variance Estimates for Full Model of Plasma IL-1 beta in EPS Case Control Study

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 3: casecontrolnumber	var(cons)	0.753702	0.731585	-0.68018	2.187582
Level 2: id	var(cons)	0.984881	0.753065	-0.4911	2.460861

11.5.3.9 Full Model for D/P Cr in EPS Case-Control Study

Table 11-66: Level Summary for Full Model of D/P Cr in EPS Case Control Study

		Observations per Group		
Level Variable	Groups	Minimum	Maximum	Average
casecontro~r	11	3	25	13.4
id	38	1	11	3.9

Log likelihood	127.0728
Deviance	-254.146

Table 11-67: Coefficients for Full Model of D/P Cr in EPS Case Control Study

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
eps	0.024151	0.0399176	0.61	0.545	-0.05409	0.102389
ttillendyear	0.034722	0.0060311	5.76	0	0.022901	0.046542
ageatpdfin~h	-0.00166	0.0011378	-1.46	0.143	-0.00389	0.000565
cons	0.931729	0.0726756	12.82	0	0.789287	1.07417

Table 11-68: Variance Estimates for Full Model of D/P Cr in EPS Case Control Study

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 3: casecontrolnumber	var(cons)	0	0	0	0
Level 2: id	var(cons)	0.0088881	0.002596	0.0038	0.013977
Level 1: samplenumber	var(cons)	0.0067799	0.000916	0.004984	0.008576

11.5.4 Chapter 8 Results

11.5.4.1 Competing Risks of EPS

Competing-risks regression	No. of obs = 17504
	No. of subjects = 17504
Failure event : event == 1	No. failed = 100
Competing event: event == 2	No. competing = 9479
	No. censored = 7925
	Wald chi2(5) = 162.30
Log pseudolikelihood = -799.98746	Prob > chi2 = 0.0000

Table 11-69: Coefficients for Competing Risks Model of EPS

	SHR	Robust SE	z	P>z	95% Confidence Interval	
dm	0.757647	0.223716	-0.94	0.347	0.424743	1.351472
sex	0.972065	0.195825	-0.14	0.888	0.654966	1.442686
age	0.957651	0.00548	-7.56	0	0.946971	0.968452
3.ds	6.865962	1.44182	9.17	0	4.549389	10.36215
diseasecode	0.504925	0.129552	-2.66	0.008	0.305371	0.834882

11.5.4.2 Cox Model for EPS

No. of subjects = 17504 Number of obs = 17504

No. of failures = 100

Time at risk = 14524311

LR chi2(5) = 91.13

Log likelihood = -680.88427 Prob > chi2 = 0.0000

Table 11-70: Coefficients for Cox Model of EPS

	HR	Std. Err.	z	P>z	95% Confidence Interval	
dm	1.472894	0.417053	1.37	0.171	0.845574	2.565613
sex	1.292691	0.267005	1.24	0.214	0.862342	1.937805
age	0.977165	0.006702	-3.37	0.001	0.964117	0.990391
3.ds	8.585785	1.888924	9.77	0	5.578413	13.21446
diseasecode	0.589214	0.142229	-2.19	0.028	0.367116	0.945677

11.5.4.3 Cox Model for Death

No. of subjects = 17504 Number of obs = 17504

No. of failures = 9479

Time at risk = 14524311

LR chi2(5) = 2380.05

Log likelihood = -81546.141 Prob > chi2 = 0.0000

Table 11-71: Coefficients for Cox Model of Death

	HR	Std. Err.	z	P>z	95% Confidence Interval	
dm	1.430263	0.033193	15.42	0	1.366663	1.496823
sex	1.026139	0.021424	1.24	0.217	0.984996	1.069
age	1.030916	0.000873	35.95	0	1.029206	1.032628
3.ds	1.185559	0.048675	4.15	0	1.093895	1.284903
diseasecode	1.347782	0.035187	11.43	0	1.280552	1.418542

11.5.5 Chapter 9 Results

11.5.5.1 Multilevel Model for Reciprocal of Random blood glucose in Non-Diabetic Incident Patients

Table 11-72: Level Summary for Model of Reciprocal of Random blood glucose in Incident Patients

		Observations per Group		
Level Variable	Groups	Minimum	Maximum	Average
cent	10	2	80	28.6

Log likelihood	507.3061
Deviance	-1014.61

Table 11-73: Coefficients for Model of Reciprocal of Random blood glucose in Incident Patients

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
totdayglu	0.000133	7.76E-05	1.71	0.087	-1.9E-05	0.000285
korean	-0.02712	0.013399	-2.02	0.043	-0.05338	-0.00086
bmi	-0.00084	0.000564	-1.5	0.134	-0.00195	0.000261
age	-0.00071	0.000164	-4.34	0	-0.00103	-0.00039
bpsys	-0.00013	0.000121	-1.08	0.281	-0.00037	0.000107
dpcr	0.035501	0.025477	1.39	0.163	-0.01443	0.085435
lengthpd	-0.0002	0.000139	-1.43	0.154	-0.00047	7.42E-05
alb	0.000133	0.000617	0.22	0.829	-0.00108	0.001342
sodium	0.001989	0.000712	2.79	0.005	0.000593	0.003385
logpil6	-0.00279	0.00847	-0.33	0.742	-0.01939	0.01381
urinevol	1.84E-06	3.72E-06	0.5	0.62	-5.45E-06	9.14E-06
comorbscore	-5.5E-05	0.003491	-0.02	0.987	-0.0069	0.006786
icodex	0.007697	0.007735	1	0.32	-0.00746	0.022858
cons	-0.04663	0.10518	-0.44	0.658	-0.25278	0.159519

Table 11-74: Variance Estimates for Model of Reciprocal of Random blood glucose in Incident Patients

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 2: cent	var(cons)	0.000262	0.000154	-3.9E-05	0.000564
Level 1: id	var(cons)	0.001597	0.000136	0.001331	0.001863

11.5.5.2 Multilevel Model for Reciprocal of Random blood glucose in Non-Diabetic Prevalent Patients

Table 11-75: Level Summary for Model of Reciprocal of Random blood glucose in Prevalent Patients

		Observations per Group		
Variable	Groups	Minimum	Maximum	Average
cent	10	2	57	20.9

Log likelihood	375.969
Deviance	-751.938

Table 11-76: Coefficients for Model of Reciprocal of Random blood glucose in Prevalent Patients

	Coef.	Std. Err.	z	P>z	95% Confidence Interval	
totdayglu	-0.00031	8.21E-05	-3.72	0	-0.00047	-0.00014
korean	-0.01055	0.020292	-0.52	0.603	-0.05032	0.029219
bmi	-0.00061	0.0007	-0.87	0.382	-0.00198	0.000761
age	-0.0004	0.000193	-2.05	0.04	-0.00077	-1.8E-05
bpsys	0.000204	0.000136	1.5	0.134	-6.3E-05	0.000471
dpcr	-0.02408	0.027952	-0.86	0.389	-0.07886	0.030706
lengthpd	-5.94E-07	4.59E-06	-0.13	0.897	-9.60E-06	8.41E-06
alb	0.001061	0.000716	1.48	0.139	-0.00034	0.002465
sodium	0.001511	0.000862	1.75	0.08	-0.00018	0.003201
logpil6	-0.01008	0.012198	-0.83	0.408	-0.03399	0.013822
urinevol	-1.76E-06	5.54E-06	-0.32	0.751	-1.3E-05	9.09E-06
comorbscore	0.003857	0.003466	1.11	0.266	-0.00294	0.01065
icodex	-0.14915	0.043924	-3.4	0.001	-0.23524	-0.06306
icobyglu	0.001963	0.000634	3.1	0.002	0.000721	0.003204
icobyglu2	-5.17E-06	2.10E-06	-2.47	0.014	-9.28E-06	-1.06E-06
cons	-0.00409	0.123332	-0.03	0.974	-0.24581	0.237639

Table 11-77: Variance Estimates for Model of Reciprocal of Random blood glucose in Prevalent Patients

Random-effects	Parameters	Estimate	Std. Err.	95% Confidence Interval	
Level 2: cent	var(cons)	0.000741	0.000389	-2.1E-05	0.001503
Level 1: id	var(cons)	0.001446	0.000145	0.001162	0.00173

11.5.6 Cox Survival Model for Non-Diabetic Incident Patients

No. of subjects = 303 Number of obs = 303

No. of failures = 109

Time at risk = 20525.04314

Wald chi2(9) = 82.18

Log pseudolikelihood = -288.35275 Prob > chi2 = 0.0000

Table 11-78: Coefficients for Cox Survival Model in Non-Diabetic Incident Patients

_t	HR	Robust SE	z	P>z	95% Confidence Interval	
age	1.066915	0.012714	5.44	0	1.042284	1.092128
logpil6	3.074614	0.925275	3.73	0	1.704628	5.545638
comorbscore	1.479189	0.172078	3.37	0.001	1.177609	1.858002
urinevolli~e	0.800935	0.120475	-1.48	0.14	0.596432	1.075556
lengthpdmo~h	1.135769	0.28154	0.51	0.608	0.698699	1.846248
alb	0.95664	0.021496	-1.97	0.049	0.915423	0.999713
dpcr10	1.052437	0.075537	0.71	0.476	0.914329	1.211405
glucat						
1	0.947671	0.225575	-0.23	0.821	0.594354	1.51102
2	1.151431	0.403989	0.4	0.688	0.578879	2.290281

11.5.7 Cox Survival Model for Non-Diabetic Prevalent Patients

No. of subjects = 232 Number of obs = 232

No. of failures = 97

Time at risk = 502483

Wald chi2(9) = 130.66

Log pseudolikelihood = -244.84819 Prob > chi2 = 0.0000

Table 11-79: Coefficients for Cox Survival Model for Non-Diabetic Prevalent Patients

_t	HR	Robust SE	z	P>z	95% Confidence Interval	
age	1.059133	0.009953	6.11	0	1.039805	1.078821
logpil6	3.08304	1.457272	2.38	0.017	1.220783	7.786094
comorbscore	1.392261	0.13807	3.34	0.001	1.146324	1.690962
urinevolli~e	0.646956	0.152002	-1.85	0.064	0.408212	1.025331
lengthpdmo~h	1.012945	0.005116	2.55	0.011	1.002966	1.023022
alb	0.974382	0.024285	-1.04	0.298	0.927929	1.02316
dpcr10	1.237036	0.121545	2.16	0.03	1.020346	1.499743
glucat						
1	1.454432	0.360257	1.51	0.13	0.895064	2.363376
2	1.323676	0.611125	0.61	0.544	0.535541	3.271682

11.6 Appendix F - Ethics Approval

Multi-Centre Research Ethics Committee for Wales

MREC for WALES

Pwyllgor Ymchwil Ethegau Aml-Ganolfan yng Nghymru

Chairman/Cardeirydd: Dr John Saunders

Administrator/Gweinyddes: Corinne Scott

Temple of Peace and Health, Cathays Park, Cardiff CF10 3NW
Teml Heddwch ac Iechyd, Parc Cathays, Caerdydd CF10 3NW

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MREC website: http://ds.dial.pipex.com/mrec
e-mail : corinne.scott@bro-taf-ha.wales.nhs.uk

Dr. Nicholas Topley,
Institute of Nephrology,
University of Wales College of Medicine,
Heath Park,
Cardiff CF14 4XN

April 16th 2002

Dear Dr. Topley,

Research Protocol MREC 02/9/14 **(Please quote this in all correspondence)**
Longitudinal evaluation of peritoneal membrane function, inflammation and structural integrity in peritoneal dialysis

I have reviewed the documents submitted in response to the MREC for Wales decision made at its meeting held on April 16th 2002, and set out in our letter dated April 16th 2002.

The documents reviewed were as follows:
(By full Committee)
- Application Form including Annexe C
- Full Protocol and references
- ~~Patient Information Sheet, version 1.1 dated March 16th 2002~~ **Superseded**
- ~~Patient Consent Form~~ **Superseded**
- GP letter
- Curriculum Vitae for Principal Researcher, Dr. Nicholas Topley

(By Chairman)
- Patient Information Sheet and Consent Form, version 1.2 dated April 8th 2002

As Chairman, acting under delegated authority, I am satisfied that these accord with the decision of the Committee and agree that there is no objection on ethical grounds to the proposed study. I am, therefore, happy to give you our approval on the understanding that you will follow the conditions of approval set out below. A full record of the review undertaken by the MREC is contained in the attached Response Form. The project must be started within three years of the date on which MREC approval is given.

- You must follow the protocol agreed and any changes to the protocol will require prior MREC approval.
- If projects are approved before funding is received, the MREC must see, and approve, any major changes made by the funding body. The MREC would expect to see a copy of the final questionnaire before it is used.
- You must promptly inform the MREC of:

(i) deviations from or changes to the protocol which are made to eliminate immediate hazards to the research subjects;
(ii) any changes that increase the risk to subjects and/or affect significantly the conduct of the research;
(iii) all adverse drug reactions that are both serious and unexpected;
(iv) new information that may affect adversely the safety of the subjects or the conduct of the trial.

- You must complete and return the standard progress report form to the MREC one year from the date on this letter and thereafter on an annual basis. This form should also be used to notify the MREC when your research is completed.

While the MREC has given approval for the study on ethical grounds, it is still necessary for you to obtain management approval from the relevant Clinical Directors and/or Chief Executive of the Trusts (or Health Boards/HAs) in which the work will be done.

LREC Review

When undertaking the review of your project the MREC observed that this study falls under the Supplementary Operational Guidelines for NHS Research Ethics Committees, published in November 2000. This study is classed as Category D research, and therefore does not require LREC review.

For this reason you are asked to only inform the appropriate LREC of the project by sending a copy of this letter and also **giving the name and contact details of the local clinician involved**. If (unusually) the LREC has any reason to doubt that the local clinician is competent to carry out the tasks required, it will inform the clinician and the MREC that gave ethical approval giving full reasons.

You are not required to wait for confirmation from the LREC before starting your research.

Whilst the MREC would like as much information as possible about local sites at the time you apply for ethical approval it is understood that this is not always possible. You are asked, however, to send details of local sites as soon as a researcher has been recruited. This is essential to enable the MREC to monitor the research it approves.

The MRECs are fully compliant with the International Conference on Harmonisation/Good Clinical Practice (ICH GCP) Guidelines for the Conduct of Trials Involving the Participation of Human Subjects as they relate to the responsibilities, composition, function, operations and records of an Independent Ethics Committee/Independent Review Board. To this end it undertakes to adhere as far as is consistent with its Constitution, to the relevant clauses of the ICH Harmonised Tripartite Guideline for Good Clinical Practice, adopted by the Commission of the European Union on 17 January 1997. The Standing Orders and a Statement of Compliance were included on the computer disk containing the guidelines and application form and are available on request or on the Internet at http://dspace.dial.pipex.com/mrec.

Yours sincerely,

Dr. John Saunders
Chairman
MREC for Wales

ENCS : MREC Response Form and Attendance List for MREC Meeting of April 11th 2002.

MULTI-CENTRE RESEARCH ETHICS COMMITTEE FOR WALES

RESPONSE FORM

1	**Details of Applicant**
	Dr. Nicholas Topley, Institute of Nephrology, University of Wales College of Medicine, Heath Park, Cardiff CF14 4XN
2	**Title of Project**
	Longitudinal evaluation of peritoneal membrane function, inflammation and structural integrity in peritoneal dialysis
3	**Name of Sponsor**
	None
4	**Details of MREC**
	MREC for Wales, Temple of Peace & Health, Cathays Park, Cardiff, CF10 3NW.
5	**MREC Reference Number**
	02/9/14
	YOUR APPLICATION HAS BEEN CONSIDERED BY THE MREC FOR WALES WHO MADE THE FOLLOWING COMMENTS :
1	**Qualifications of the Applicant**
	No comments
2	**Scientific Value and Validity of the Proposal**
	No comments
3	**The Welfare of the Research Subject**
	No comments

4	**Patient Information Sheet**
	The PIS should state the following : 1) That the subject is free to withdraw from the study at any time 2) the subject will not benefit from participation in the study 3) the subject's GP will be contacted to inform them of their participation in the study 4) there should be some comment regarding the anonymisation of the individual's data Consent form should : 1) give permission for the subject's GP to be contacted ***The revised Patient Information Sheet was received on April 16th 2002. This has been reviewed and approved by the Chairman of the MREC for Wales, Dr. John Saunders.***
5	**Confidentiality**
	No comments
6	**General Comments**
	The need for LREC review This research falls under Category D of the Guidelines for Epidemiological Research and no LREC approval is therefore required.

REVIEW BY THE MREC

The following items have been reviewed by the MREC for Wales in connection with the above study to be conducted by the above researcher :

Protocol	
Investigators Drug Brochure	n/a
Patient Information Sheet and Consent Form, version 1.2 dated April 8th 2002	
GP letter	
CTX	n/a
Protocol amendment	n/a
Methods of initial recruitment to study	
Compensation arrangements for subjects	n/a
Payments to researcher	n/a
Provision of expenses for subjects	n/a

Your application has been approved.

Date of review : April 11th 2002
Date of approval : April 16th 2002

Signature of Chairman ..... **Date** .....

MREC FOR WALES

Attendance List for the MREC for Wales' meeting on April 11th 2002

Dr John Saunders	Chairman	Professional (Hospital Consultant)
Dr. Gordon Taylor	Vice Chairman	Lay member
Dr. Barbara Bale		Professional (Midwife)
Dr. Peter Beck		Professional (Hospital Consultant)
Dr. Alison George		Lay member
Mrs. Phillipa Herbert		Lay member
Dr. Mohammad Obaidullah		Professional (GP)
Mr. Simon Rivers		Professional (Pharmacist)
Dr. Paul Wainwright		Professional (Nurse)

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